HPLC SEPARATION OF AMINES WITH A ZIRCONIA-BASED COLUMN COUPLED TO A GAS-PHASE CHEMILUMINESCENCE NITROGEN SPECIFIC DETECTOR (CLND)

A Dissertation

by

SILVIA ADRIANA SALINAS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2004

Major Subject: Chemistry

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Approved as to style and content by:

Gyula Vigh (Chair of Committee)	Emile A. Schweikert (Member)
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ABSTRACT

HPLC Separation of Amines with a Zirconia-Based Column Coupled to a Gas-Phase Chemiluminescence Nitrogen Specific Detector (CLND). (May 2004)

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Gas-phase chemiluminescence nitrogen specific detector (CLND) is used for the direct analysis of underivatized nitrogen-containing components such as alkylamines that cannot be detected by the so-called universal HPLC detector, the UV detector.

However, alkali metal hydroxides cannot be used as mobile phase constituents with the CLND because they form non-volatile particulate combustion products that foul the detector. Therefore, trimethylsulfonium hydroxide (TMSOH) has been selected as a strong base for use with the CLND, because its combustion products, CO_2, H_2O and S_xO_y, are volatile. An alkali-stable zirconia-based column was used and coupled to the CLND. Zirconia-based columns are mechanically and hydrolytically more stable than silica-based columns, which have a working pH range from 3 to 8 only. Zirconia-based columns can be used at a pH from 1 to 14 and can be used at temperatures up to 200 C.

The separation of amines was carried out at high pH values where the amino groups were deprotonated. Primary, secondary, tertiary and quaternary amines were separated using a pH=13.7 mobile phase that contained only TMSOH, methanol and

water. Good peak shapes were observed for all, except n-alkylamines and samples that contained both amino groups and alcohol groups.

DEDICATION

Quiero dedicar este trabajo a las personas mas importantes en mi vida que son mis papas. Por el respeto y apoyo que me han demostrado siempre, les debo quien soy y espero que algun dia puedan estar tan orgullosos de mi como yo lo estoy de ellos.

A mis hermanos Raul y Angeles, a mi tia Lupita, y a Pily por todo su apoyo.

A mi abuelita Ernestina, porque siempre esta conmigo y porque creo que estaria orgullosa de mi si viviera.

Y finalmente a la persona que me mantuvo de pie durante mis estudios de doctorado, mi esposo Marcus Moreno.

ACKNOWLEDGEMENTS

I want to thank my advisor Professor Gyula Vigh, for all the knowledge shared in the last four years and for all his help during my stay in his lab. I would also like to thank my committee members Dr. David H. Russell, Dr. Marian Hyman and Dr. Edward Funkhouser for all their comments and suggestions at any point during my studies. I have to thank everyone in the Separation Science Group for all their help and their friendship. I must thank my group of friends, “the Pachucos” for being a family to me in a strange country.

Quiero agradecer a mis padres Raul y Angeles, por haberme apoyado siempre, a mis hermanos, Raul, Claudia, Angeles y Gabriel por haber completado mi vida incluyendo a Ornella, Gabi y Emiliano y sobre todo a Regina, por habernos enseñado tanto en tan poco tiempo, siempre te voy a recordar. A Julie Wilson por toda su ayuda durante todo este tiempo. A Omar, Rosario y Silvia, por haberme prestado su mano y hombro cada vez que los necesite. A Marian Hyman, MC, Caroline, Joanie y Liliana, por haberme mantenido cuerda todo este tiempo. Finalmente quiero agradecer a Marcus por haber entrado a mi vida, hacerme tan feliz y por toda su paciencia.

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INTRODUCTION

1.1 Fundamentals of HPLC

High performance liquid chromatography (HPLC) is one of the most common separation techniques used. HPLC separates analytes by means of different interactions between the stationary and mobile phases. HPLC is divided into two main separation modes: reversed-phase HPLC (RP-HPLC) and normal-phase HPLC (NP-HPLC). In NP-HPLC mode, the stationary phase is more polar than the mobile phase and in reversed phase, the stationary phase is more hydrophobic than the mobile phase. RP-HPLC is most commonly used because neutral samples as well as ionic samples can be separated.

Some of the important parameters in HPLC are retention factor (k'), which indicates the retention of the analyte on the stationary phase with respect to the non-retained component [1] and is calculated by the following equation

$$k' = (t_r - t_o) / t_o \qquad (1)$$

where t_r is the retention time of the analyte and t_o is the column dead time, which corresponds to the elution time of the non-retained component.

This dissertation uses the style and format of Journal of Chromatography A.

Plate number (N), is a measure of column quality or column performance [1] and is calculated using equation 2

$$N = 5.54(t_r/W_{1/2})^2 \qquad (2)$$

where $W_{1/2}$ is the width of the peak at half height.

Another important parameter in this investigation is peak asymmetry factor (A_s). Peak asymmetry factor is used to determine whether a peak shape is symmetric or not. The peak asymmetry is measured at 10% of full peak height as shown in Figure 1 and the relationship used is shown in equation 3.

$$As = B/A \qquad (3)$$

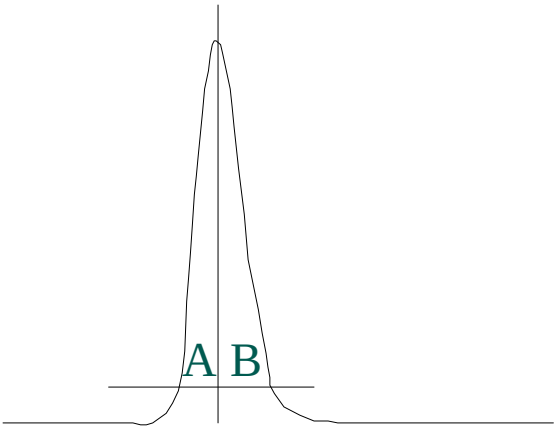


Figure 1. Determination of peak asymmetry.

A peak asymmetry value lower than 1.5 indicates that the peak is symmetric.

Most of the RP-HPLC columns available are silica-based columns. The most common silica based columns used in RP-HPLC contain octyl (C8) and octadecyl (C18) groups bonded to silica. Even after derivatization, up to 30% of the silanol groups of silica remain unreacted [1]. These silanol groups are undesirable acidic sites and can be troublesome when bases are to be separated.

However, the main disadvantage of silica-based columns is its low chemical stability. At pH lower than 3 the siloxane linkages are attacked hydrolytically and the bonded phases are removed from the surface [1-5]. At pH higher than 8 the silica is dissolved [1-5]. Because of this, different metal oxides have been studied as stationary phases to replace silica. Some of these metal oxides are alumina [6-9], titanium [10-11] and zirconia [10-11]. Zirconia-based columns have been studied with favorable results. It has been found that zirconia-based columns are hydrochemically stable from pH 1 to 14 and can be used up to 150ºC [12-29].

1.2 HPLC Using Zirconia-Based Columns

Zirconia-based stationary phases are made of zirconium atoms with bonds to seven oxygen atoms [9]. This is what makes zirconia-based columns more stable than silica-based columns, where silicon is only bonded to four oxygen atoms [9]. At the surface of zirconia some of the zirconium-oxygen bonds are interrupted leaving some positive charges exposed, which act as hard Lewis acid sites [9]. Due to a high concentration of these Lewis sites that can interact with analytes, the zirconia surface has been covered with polymers to try to eliminate interaction between analytes and the

Lewis acid sites on the stationary phase. Different zirconia-based columns are commercially available, such as anion exchange, cation exchange, normal phase, mixed mode ion ligand exchange and reversed-phase columns [9]. The coated polymer on the zirconia surface used in this research is polybutadiene (PBD). Even when the zirconia surface has been modified with PBD, there are still some Lewis acid sites exposed. [30-42].

It has been reported that hard Lewis bases such as hydroxide ion [9, 30-32], fluoride ion [30-31, 39], sulfonate ion [30-31] and acetate ion [30-31] interact with zirconia-based columns and have binding constants of $10^{14.3}$, $10^{8.8}$, $10^{3.7}$ and $10^{3.35}$, respectively. Nitrate and chloride ions form weak complexes with zirconia [9,30-31] and they both have binding constants of $10^{0.3}$. Even though carboxylate ion binds relatively weakly with zirconia, the complexation strength increases as the number of carboxylate groups increases per analyte. An example is the binding constant of acetate ion and zirconia, $K=10^{0.3}$ and the binding constant of oxalate ion and zirconia, $K=10^{9.8}$ [31].

Due to the strong binding of fluoride ion and phosphate ion to zirconia, these hard Lewis bases have been used in mobile phases. This has been done because the ions compete for the Lewis acid sites of zirconia with the sample analytes, thus decreasing the interaction of the analytes with the surface and improving their peak shape [30-32, 37-42]. In the present investigation hydroxide ion is used in the mobile phase with concentrations as high as 500 mM as replacement of fluoride and phosphate ions.

1.3 Detection of UV Transparent Nitrogen Containing Species

UV absorbance detector is the most commonly used detector in HPLC, however, the analyte must have a chromophore group in its structure to be detected. UV transparent analytes have to be derivatized for their detection by a UV absorbance or fluorescent detector. Amino acids and UV transparent amines are some of the species that have to be derivatized for UV absorption or fluorescent detection. Some of the most common derivatizing reagents used in UV absorbance or fluorescent detection are o-phthaldialdehyde (OPA) [42-46], dansyl chloride [47-48], N-hydroxysuccinimidyl fluorescein-O-acetate (SIFA) [49-50], 1,2-naphthoquinone-4-sulfonate (NQS) [51], salicylaldehyde-5-sulfonate (SAS) [52-53], 3,5-dinitrobenzoyl (DNB) [54], 2,4,6-trinitrobenzenesulfonic acid [55], 5-furoylquinoline-3-carboxaldehyde (FQ) [56], 4-chloro-7-nitrobenzofurazan [57] and phthalic anhydride [58].

However, most derivatization methods have disadvantages, e.g., the product obtained after derivatization with SAS are not stable and decompose at high pH, the reaction takes up to 4.5 hrs [52-53] and the derivatizing reagent can react only with primary and secondary amines. NQS and DNB have been also used, however they can also react with primary and secondary amino groups and NQS is not stable at high pH. SIFA can only be reacted at high pH.

1.4 Chemiluminescence

Chemiluminescence (CL) is the emission of electromagnetic radiation as a result of

a chemical reaction [59-60]. CL is a dark field technique, without strong background light levels, which reduces the noise signal and leads to improved detection limits. The reaction involved in CL is almost invariably an oxidation reaction. CL is a fluorescence mechanism (spin allowed, radiative deactivation of the lowest excited singlet state) from the electronically excited product of a chemical reaction. The radiative transition between excited and ground states of the same spin occurs in a time frame of 10^{-11} to 10^{-7} s after excitation, this process is called fluorescence. The only difference between CL and fluorescence is that the latter is produced when light-emitting molecules are electronically excited by the absorption of light while in CL no external light is employed.

When excitation occurs, the molecule is transferred to an excited singlet state, vibrational relaxation carries the molecule to the lowest vibrational level of the lowest excited singlet state. Once the molecule is in its lowest excited singlet state, the molecule has to come down to its ground electronic state. However, only a fraction of the molecules formed in the lowest excited singlet state actually fluoresce. $ö_{CL}$ is the quantum yield or fluorescence efficiency which value is usually a fraction but may be unity in some rare cases.

$ö_{CL}$ is the probability (rate constant) of fluorescence (K_f) and competitive processes (K_d) expressed as

$$ö_{CL} = K_f / (K_f + K_d) \qquad (3)$$

the greater Kd, the lower the value of $ö_{CL.}$ $ö_{CL}$ determines the intensity of chemiluminescence observed as a result of a particular reaction.

Another important parameter in CL is $\hat{o}_{CL}$, which is the lifetime of the lowest excited singlet state. $\hat{o}_{CL}$ and $\ddot{o}_{CL}$ are related by the following equation

$$\hat{o}_{CL} = \ddot{o}_{CL} * \hat{o}_{N} \quad (4)$$

where $\hat{o}_{N}$ is the natural lifetime of the excited state which represents the lifetime the fluorescing molecule would have if fluorescence was the only pathway for deactivating the lowest excited singlet state. $\hat{o}_{N}$ is only a function of the molecular structure.

The intensity of the CL (I_{CL}) emission produced depends on the efficiency of generating molecules in the excited state. I_{CL} is related to the quantum yield or quantum efficiency and the rate of the reaction as shown below

$$I_{CL} = \ddot{o}_{CL} * -dA/dt \quad (5)$$

where I_{CL} is the CL emission intensity (in photons/second) and -dA/dt is the rate at which the CL precursor of A is consumed. Most CL reactions used for analytical purposes have a $\ddot{o}_{CL}$ of 0.001 to 0.1, even smaller values can be used due to the almost complete absence of background light.

There are two general mechanisms of CL, direct and indirect CL. In the direct CL, two reagents (substrate and oxidant and sometimes a catalyst) react to form a product or an intermediate and a fraction of the product is formed in an electronically excited state that relaxes to ground state with emission of a photon. The substrate is usually the CL precursor and is converted into an electronically excited molecule, which is responsible for the emission of the photon. The catalyst reduces the activation energy and

provides the adequate environment for a high CL efficiency. In the indirect mechanism there is a transfer of energy from the excited molecule to a fluorophore. In this way, molecules that are unable to participate in a CL reaction become detectable.

CL detection can be done in one of two phases, in liquid phase or in the gas phase. In liquid phase, two of the most commonly used CL reactions are luminol [61-64] and $Ru(bipy)_3^{3+}$ [61, 65-68]. In these reactions however, the separation medium has to be compatible with the CL reaction, which may be difficult to achieve in some cases. In the CL reaction with luminol, an oxidizer, usually hydrogen peroxide is required for its oxidation and a transition metal ion complexing agent is added to form an excited state complex, which emits photons [61]. The analyte is detected when complexing with the transition metal ion thereby disrupting the formation of the chemiluminescencing complex, which causes a decrease in the emitted light intensity. $Ru(bipy)_3^{3+}$ on the other hand, is used in an electrochemiluminescence reaction where the analyte participates in the redox reaction to create the chemiluminescent species [61].

The most common gas-phase CL reactions involve phosphorus [69-71], sulfur [72-74] and nitrogen [75-76]-containing species. The CL reactions of sulfur and nitrogen-containing species are well known while the CL reaction of phosphorus-containing species is not yet completely understood.

The sulfur-containing species is oxidized to form sulfur dioxide, which is then reduced with hydrogen to form sulfur monoxide, which is subsequently reacted with ozone to form sulfur dioxide in the excited state. This then relaxes to the ground state and emits photons. The nitrogen-containing species is oxidized to form nitrogen monoxide,

which is then reacted with ozone to form nitrogen dioxide in the excited state, which then decays by emission of a photon [72-74].

1.5 CLND Instrumentation

CLND is a gas-phase chemiluminescene nitrogen specific detector. In this research, the CLND is coupled to a HPLC system; the effluent from the column is continuously introduced into a furnace held at 1050 C through a nebulizer that forms a plume of the sample and the mobile phase. Sample and mobile phase are oxidized in the furnace with a stream of oxygen and argon forming water, carbon dioxide and nitric oxide, following equation 6.

$$C_xH_yN_z + (x + ¼\ y + ½\ z)\ O_2 \rightarrow zNO + xCO_2 + y/2\ H_2O \qquad (6)$$

The NO-containing reaction mixture is then transported through a drier membrane where water is removed, and directed to the reaction chamber. In the reaction chamber NO is reacted with ozone and the reactions shown below occur:

$$NO + O_3 \rightarrow NO_2^* + O_2 \qquad (7)$$

$$NO + O_3 \rightarrow NO_2 + O_2 \qquad (8)$$

$$NO_2^* + M \rightarrow NO_2 + M \qquad (9)$$

$$NO_2^* \rightarrow NO_2 + hí \qquad (10)$$

where M represents a colliding body.

CLND directly detects nitrogen-containing compounds even if they do not contain chromophores. This is an advantage that has not been utilized to the fullest. One of the advantages of the CLND over any other detector used in the separations area is the use of a universal calibration curve.

A problem encountered with any CLND system however, is the use of buffers containing alkali metals and any buffer constituent with non-volatile combustion products. Buffer constituents with non-volatile combustion products are oxidized in the furnace and particles of non-volatile oxides are formed. These particles remain in the system fouling the detector.

1.6 Reviewed Literature

Papers regarding the applications of the HPLC-CLND system include Fujinari and coworkers, that in 1992 introduced the CLND showing the direct detection of ammonium ion in water using ion chromatography [77,78]. In 1994, Fujinari and Manes demonstrated the separation and detection of underivatized peptides from casein hydrolysate and other hydrolysate-based foods [79]. Amino acids, peptides and proteins have also been analyzed [80, 81], caffeine in different drinks [82] as well as aspartame and saccharin [83]. Red hot peppers have also been analyzed for capsaicin and dihydrocapsaicin, which are responsible for the hot flavors of peppers [84, 85], nucleotides, nucleosides [86] and isomers of aniline [87]. Fujinari pointed out that diatomic nitrogen or molecules that form it in their decomposition process such as azides do not react with ozone, therefore can not be detected using the CLND. One of the

important aspects about this paper was that Fujinari introduced the CLND as an additional detector to complete the analysis of samples that contain both nitrogen containing species and nitrogen-free species [88]. Supercritical fluid chromatography coupled to a CLND was first introduced in 1996 by Shi et al. They found equimolar responses for different nitrogen-containing species and a linear range of response of up to three orders of magnitude [89, 90, 91]. In 1997, size-exclusion chromatography was successfully coupled to a CLND by Fujinari and coworkers and the CLND was also demonstrated as an additional detector required for the complete detection of complex mixtures [92, 93].

In 1998, Taylor and coworkers introduced the CLND in addition to UV and MS (mass spectrometry) as a tool to help in the characterization of samples in combinatorial chemistry. It was mentioned by the authors that CLND has brought about the missing piece, quantification of parent ions and impurities, which is difficult to assess using ELSD (evaporative light scattering detector) since it has a different response for each analyte. The linear response of the CLND was equivalent for the compounds tested and response was independent of the mobile phase composition indicating that CLND is indeed a universal quantitative detector for nitrogen-containing species [94, 95, 96]. Shah and coworkers have corroborated the CLND's contribution to combinatorial chemistry. UV, MS and even ELSD are used for qualitative analysis while CLND as a means of estimating compound concentration. They corroborated the linear response of CLND for 78 compounds [97].

Brannegan and coworkers studied ethoxyquin and its related bases in 2001 using the CLND [98]. The objective of their research was to evaluate the equimolarity,

linearity, linear range and limit of detection of the HPLC-CLND system. The equimolarity was observed, linearity was found to be of three orders of magnitude and the limit of detection they found was 25 pmol nitrogen [98]. In that same year, Lucy and coworkers, analyzed the detection of nitrate and nitrite ions using ion chromatography. It was stated that even though the bond dissociation energy of cyanide (891 kJ/mol) was very close to that of nitrogen gas (941.4 kJ/mol), cyanide does react with oxygen gas and produces NCO and radical O. The radical O reacts with NCO to yield CO and NO, which is then carried to the reaction chamber to react with ozone and be detected. The N-H bond dissociation energy in an amine is 393 kJ/mol [99]. Petritis and coworkers showed the separation and detection of underivatized amino acids using HPLC-CLND utilizing a mobile phase with perfluorinated carboxylic acid as the ion-pairing agent. The amino acids were tested in serum, tobacco extract and wine using a single calibration curve. The limits of detection found were from 2.5 to 7.5 ìM [100].

Nussbaum and coworkers, in 2002, demonstrated that quantitation of components is difficult with a detector any other than CLND, as the response factors differ significantly from one sample to another. MS detectors are universal, but their response per unit weight depends on the ionization process. One of the samples tested by the authors is nifedipine and its derivative 4-(2’-nitrosophenyl)-pyridine. Both products have different UV responses at most wavelengths. However, CLND allows the determination of both compounds [101]. Petritis and coworkers compared the detecion of underivatized amino acids with the different detectors available such as ELSD, nuclear magnetic resonance spectrometer (NMR), conductivity detector, refractive index, UV, MS and CLND [102]. Their conclusion was that CLND is the only detector that allows

equimolarity for the amino acids detected [102]. The analysis of zwitterionic and cation surfactants was done by Harrison and coworkers in 2002 [103]. The analysis was done using a cyano-silica column and $BaCl_2$ as a modifier instead of transparent alkylamines as is normally done, to prevent interaction of amines with the free silanol groups. The conclusion of their studies was that a linear range was observed even when the critical micellar concentration was exceeded [103].

1.7 Trimethylsulfonium hydroxide (TMSOH)

TMSOH is a strong electrolyte [104-128] with volatile combustion products and has been used to set the pH in the mobile phase. In the present investigation, amines were separated at high pH for the deprotonation of their amino groups and increased retention without the help of ion-pairing reagents in reversed-phase chromatography. The only column commercially available that could stand such conditions was the zirconia-based column.

The objective of this research was to demonstrate the detection of underivatized UV transparent amines using the CLND. UV transparent amines from primary to quaternary as well as some common drugs have been separated using the zirconia-based-polybutadiene (PBD) column without an ion-pairing agent.

CHAPTER II

EXPERIMENTAL

2.1 HPLC Instrumentation

All chromatographic experiments were carried out on a Series1100 HPLC system (Hewlett-Packard S.A., Wilmington, DE) equipped with an autosampler and a type 8060 M gas-phase chemiluminescence nitrogen specific detector (CLND) (Antek, Houston, TX). The HPLC system was interfaced with a Hewlett Packard 35900E Interface to a Gateway 2000 P5-120 personal computer. All data collection was performed using Chemstation, version 4.21 software and the data was processed using CAESAR (Analytical Devices, Inc., San Francisco, CA) software.

2.2 Gas-Phase Chemiluminescence Nitrogen Specific Detector (CLND)

The CLND 8060 M is equipped with a nebulizer, a furnace with a temperature set at 1050°C for complete combustion of the analytes and the mobile phase, and a reaction chamber where the NO produced mixes with a stream of ozone. Ozone is formed by passing oxygen through an arc. The CLND is connected to a diaphragm vacuum pump (Vaccubrand, Wertheim, Germany). Oxygen is used for the combustion of analytes and mobile phase components and argon is required as a diluting gas to inhibit chemiluminescence quenching due to excess oxygen colliding with NO_2^* [59-60]. The

total gas flow rate used was 330 ml/min composed of 280 ml/min of oxygen and 50 ml/min of argon. A PMT tube is used for detection of the photons emitted by NO_2^* in the reaction chamber. The temperature of the PMT is set at 5°C.

2.3 Capillary Electrophoresis (CE) Instrumentation

All electrophoresis work was done on a PACE 5510 (Beckman-Coulter, Fullerton, CA) capillary electrophoretic (CE) system. Its UV detector was set at 214 nm wavelength. The temperature used was 20 C. The capillaries used in the CE experiments were 25 ìm I.D., 150 ìm O.D., untreated, fused silica capillaries (Polymicro Technologies, Phoenix, AZ). The polyimide coating was burned off at the capillary window for analyte detection as well as at both ends of the capillary to prevent analyte adsorption. The total length of the capillary was 46 cm and the length to the detector was 39 cm. The potential applied varied from one experiment to another. The PACE was interfaced to a PS2/52 (IBM) personal computer. All data collection was performed using Gold version 8.01 (Beckman) software and the data was processed using CAESAR (Analytical Devices) software.

2.4 Ion-Exchange Chromatography (IEC)

The anion exchange column was packed with a Dowex 21K resin (Dow Chemical Co., Midland, MI) with an average particle size of 580 ìm. The column had an exchange capacity of 4.9 moles of TMSI at a 5-fold excess. The total volume of the wet resin used

in the column was 19600 ml. The diameter of the anion exchange column was 28.5 cm and its length 60 cm. The flow rate used for the anion exchange column was 1.5 L/min [129]. A small column packed with 450 ml of Dowex 21K resin was used to exchange small traces of iodide present in the trimethylsulfonium hydroxide (TMSOH) solution at a flow rate of 80 mL/min.

2.5 HPLC Conditions

The column used in this research was a ZirChrom-PBD column (ZirChrom, Minneapolis, MN), a zirconia-based column that had a polybutadiene (PBD) polymer coating on the zirconia surface. The I.D. of the column was 4.6 mm and its length 150 mm. The average pore size of the zirconia packing was 300 Å and the particle diameter was 3 ìm. All measurements were made at a flow rate of 1 ml/min. The effluent was split before entering the CLND furnace: 250 µl/min went into the furnace and the rest to waste. The injection volume used was 1 µL. The column was jacketed to control the temperature of the column at 50.5°C except when mentioned otherwise, using a MGW LAUDA (Fisher, Pittsburgh, PA) water bath. The dead volume of the column was determined using urea in methanol. The lines connecting the column to the HPLC injector and splitter were made out of PEEK tubing with an I.D. of 0.0025" and an O.D. of 1/16’’.

2.6 Materials

HPLC grade methanol was obtained from EMD Chemicals Inc. (Gibbstown, NJ). The chemicals 1-aminoindan 99%, benzyltributylammonium chloride 98 %, benzyltriethylammonium chloride 99 %, benzyltrimethylammonium bromide 97 %, 1,4-butanediol 99 %, n-butylbenzylamine 98 %, butyldimethylamine 99 %, 4-chloramphetamine hydrochloride 98%, (2-(cyclohexylamino)ethanesulfonic acid 99 %, decyltrimethylammonium bromide 99 %, dibutylamine 99 %, dimethylhexylamine 98 %, ephedrine hydrochloride 99 %, ethylene glycol, halostachine (á-(methylaminomethyl)-benzyl alcohol) 99 %, 2-heptylamine 99 %, iodobutane, methanephrine hydrochloride 99 %, N-methylephedrine 98 %, methyliodide (MI), 1-(1-naphthyl)-ethylamine 98 %, norephedrine hydrochloride 99 %, octanesulfonate, sodium salt, octylamine 99 %, n-pentylamine 99 %, 2-phenylglycinonitrile hydrochloride, pindolol 97 %, (S)-(-)-2-amino-3-phenyl-1-propanol 98 %, propranolol hydrochloride 99%, tetraethylammonium bromide 99 %, tetraheptylammonium bromide 98 %, tetrahexylammonium bromide 99 %, tetrapentylammonium bromide 99 %, tetrapropylammonium bromide 98 %, tributylamine 99 %, tripropylamine 98 % and water HPLC grade were obtained from Aldrich Chemical Company (Milwaukee, WI).

Diethylamine, dimethylamine, dipropylamine, 1,6-hexanediol, 4-heptylamine, n-heptylamine, N-methylbenzylamine, octyltrimethylammonium bromide, 2-pentylamine, 3-pentylamine, trimethylsulfonium iodide (TMSI) and tripentylamine were obtained from TCI America (Tokyo, Japan).

p-Toluensulfonic acid, tris(hydroxymethyl)aminomethane and urea were obtained

from Fisher Scientific Company (Fair Lawn, NJ). Hydrochloric acid and sodium hydroxide were both of GR grade obtained from EM Science (Gibbstown, NJ).

Fenarimol, imazalil and oxyphene were obtained from Regis Technologies Inc. (Morton Grove, IL). Alprenolol hydrochloride, 6-amino-n-hexanoic acid, atenolol, bupivacaine hydrochloride, bupropion hydrochloride, chlophedianol hydrochloride, chloroquine sulfate salt, chlorpheniramine maleate salt, n-decylamine 95 %, glutamic acid, ketamine hydrochloride, ketotifen fumarate salt, labetalol hydrochloride, methoxyphenamine hydrochloride, metoprolol tartrate salt, miconazole hydrochloride, oxprenolol hydrochloride, oxybutynin hydrochloride, piperoxan hydrochloride, propafenone hydrochloride, salbutamol, tetrahydrozoline hydrochloride, terbutaline hemisulfate salt, terfenadine hydrochloride, tolperisone hydrochloride, trihexyphenidyl hydrochloride and tryptophan were obtained from Sigma Chemical Co. (St. Louis, MO).

Diltiazem hydrochloride and verapamil hydrochloride were obtained from Research Diagnostics Inc. (Flanders, NJ). Fluoxetine hydrochloride was obtained from USP (Rockville, MD). Ethyldimethylamine 99 %, dipentylamine 99 % and tetrabutylammonium iodide 98 % were obtained from Acros Organics (Pittsburgh, PA). Amphetamine and methamphetamine, both solutions of 1 mg / 1 ml of methanol were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). Benzyltripropylammonium chloride 96 % and hexyltrimethylammonium bromide 98 % were both purchased from Lancaster (Windham, NH). Butylamine was obtained from Matheson Coleman & Bell (Norwood, OH). Hexylamine 99 % was obtained from Fluka (Milwaukee, WI) and 3-heptylamine was obtained from Karl Industries (Aurora, OH). N-ethylbenzylamine was obtained from Citychemical (West Heaven, CT).

2.7 Background Electrolyte Preparation

For the characterization the residual iodide content of TMSOH a UV-transparent background electrolyte (BGE) was used. The BGE was prepared by dissolving 0.100 moles of TRIS in 500 ml of deionized water and titrating with concentrated HCl to a pH of 8.17. The electrode used in the titration was a combination glass electrode and precision pH/ion meter 150 (both from Corning Science Products, Corning, NY). The solution was quantitatively transferred to a 1L volumetric flask, the volume was brought to mark with deionized water and the pH was measured. This BGE was used for the analysis of iodide left over in the TMSOH solution. Both BGEs and the samples were filtered with a 0.45 μm pore nylon filter before use.

2.8 Preparation of HPLC Mobile Phases

The mobile phases were prepared by taking calculated volumes of TMSOH solution, water and MeOH using serologic pipettes and Fisherbrand pipettes and added to a 250 ml graduated cylinder. 100 ml of mobile phase were prepared at a time. After addition of all components, the final volume was measured and corrections for methanol and water percentages in the mobile phase were done. The mobile phases were degassed and filtered using a 0.45 μm, 25 mm diameter PTFE filter prior to use.

2.9 Ion-Exchange Procedure

The resin was pretreated by rinsing with water to remove traces of contaminants before it was used for the first time. The resin was added into a deep container, water was added, the resin was mixed, and water was decanted. This procedure was repeated until the rinse water remained clear after mixing the resin. After this, the resin was placed inside the column. The column was then rinsed with a 5-fold molar excess of sodium hydroxide and rinsed with water until the effluent from the column had a neutral pH. The resin was then rinsed with a 5-fold molar excess of hydrochloric acid and rinsed with water until the effluent from the column had a neutral pH. These two procedures were repeated twice to allow the pores of the resin to expand and contract with the different ions used. Once the pretreatment was finished, the resin was rinsed one more time with the solution necessary for the final form of the resin, either sodium hydroxide for the hydroxide form of the anion exchange resin or hydrochloric acid for the protonated form of the cation exchange resin. The resin was washed with water until the effluent of the column was neutral before the sample was added. All ion exchange experiments were carried out using a mesh on top of the resin bed to prevent its disruption and to have a higher column efficiency. Once the sample was added, the resin was washed with distilled water until the effluent from the column was neutral. At this point effluent collection was stopped. The effluent was collected in plastic bottles and capped for storage. The effluent was then concentrated using a rotavapor until the final volume was approximately 2 L. The concentration of the solution was then determined by titration using standard solutions of either phthalic acid or Tris (tris-(hydroxymethyl)-

aminomethane). The next step was to analyze the solution for any iodide left in the TMSOH solution. If the concentration of iodide was lower than 0.1%, the solution was ready for use. If the concentration was higher than 0.1 %, the ion exchange process had to be repeated until the level of impurities decreased to the desired value.

2.10 Preparation of Standards for Calibration Curve

Three stock solutions for each analyte were prepared at concentrations of 0.1 M. The standards were prepared by dissolving 0.100 moles of the analyte in 100 ml of methanol. Dilutions necessary were prepared from the stock solutions. All samples were filtered with a 0.45 ìm pore nylon filter before use.

2.11 Capillary Electrophoresis Measurements

The electrophoretic mobilities of some of the drugs studied were determined using CE. The BGE used in these experiments was the HPLC mobile phase containing 500 mM TMSOH, 5.1 % MeOH and 94.9 % H_2O. The effective mobilities were determined using pressure mediated capillary electrophoresis (PreMCE) [130]. In this method, the sample was injected and transferred into the capillary. Then a neutral marker was injected and transferred into the capillary, then another neutral marker plug was injected and transferred for the same amount of time as the other plugs. Potential was then applied for a short period of time. Another plug of neutral marker was injected and all bands were pushed out of the capillary to pass the detector window. Transfer times

and electrophoresis times were short to keep all plugs inside the capillary. The observed mobility of the analyte was determined from the change of the distance between the analyte and the neutral marker bands, before and after electrophoresis. The effective mobilities of the analytes (μ^{eff}) were obtained by subtracting the neutral marker mobility (μ^{eo}) from the observed mobility (μ^{obs}), $\mu^{eff} = \mu^{obs} - \mu^{eo}$ [130]. A typical PreMCE measurement is shown in Figure 2.

TMSOH solution was analyzed by capillary electrophoresis for its trace iodide concentration using a conventional CE method. The analyte was injected into the capillary and potential was applied until the analytes passed through the detection window.

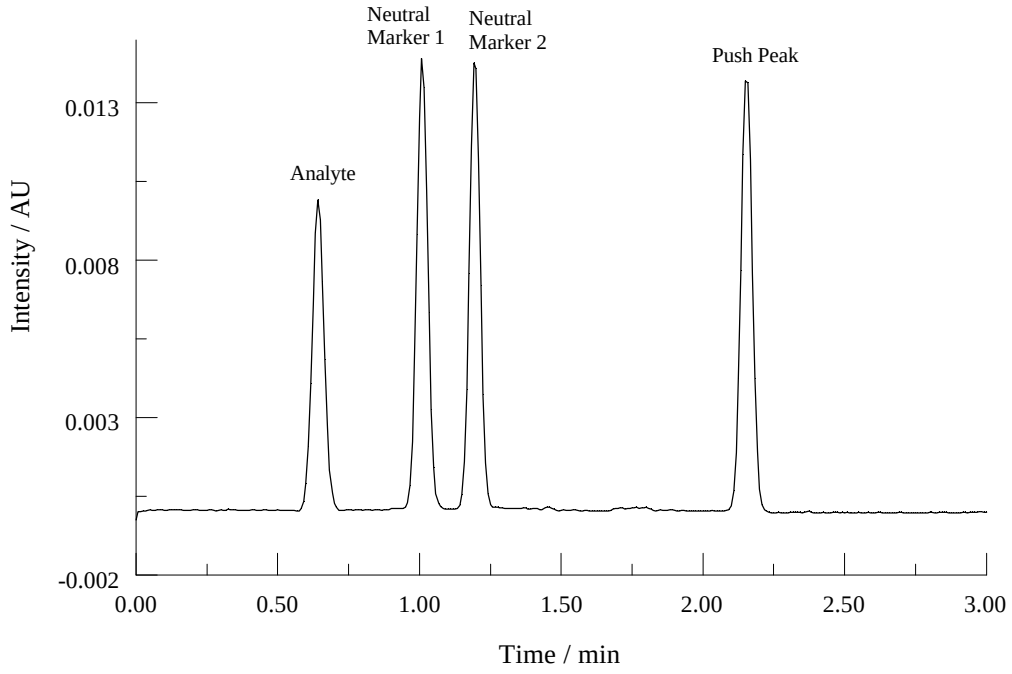


Figure 2. Three-band PreMCE mobilization profile.

CHAPTER III

RESULTS AND DISCUSSION

UV transparent amines can not be detected by a UV detector, the most widely used HPLC detector. Alkylamines have to be derivatized for UV absorbance or fluorescence detection. Most derivatization processes are time consuming, they often react with more than one functional group or the derivatized product is only stable for a short period of time and analysis of the samples has to be performed almost immediately.

An alternative to derivatization of UV transparent nitrogen-containing species is the use of a nitrogen specific HPLC detector such as the CLND.

In HPLC, the most commonly used columns are silica-based columns. However, the working pH range of silica columns is limited to 3 to 8 [1], and they are hydrothermally stable only up to 50 C [1]. Also, when weak bases are analyzed, they interact with the free-silanol groups of silica. An alternative to silica-based columns is zirconia-based columns. They have a working pH range from 1 to 14 [19-29] and are hydrothermally stable up to 200 C.

In this research, a polybutadiene (PBD) polymer-coated zirconia-based column was used. The pH of the HPLC mobile phase was set to 13.7 using an approximate final TMSOH concentration of 500 mM.

Amines are usually separated using reversed-phase ion-pair HPLC [1, 131-140] at a pH lower than the pKa of the amine. The amines are cationic at such pH, and an anionic

pairing ion is added to cause adequate retention of the amine on the reversed-phase stationary phase. In this study, the high pH of the mobile phase insured that the amines were in their neutral form and therefore, had adequate retention on a reversed-phase stationary phase.

3.1 TMSOH Concentration

Before any analyses were carried out, the optimum concentration of TMSOH was determined. One test sample of every analyte series was separated using different concentrations of TMSOH in the eluent. The concentrations used were 31.25, 62.5, 125, 250 and 500 mM TMSOH. The samples tested were tetrapropylammonium chloride, tripropylamine, dipropylamine, butylamine, sec-butylamine and 2-amino-3-phenyl-1-propanol.

As the TMSOH concentration was increased, the retention of every amine decreased except for tripropylamine, for which retention remained relatively constant at all different concentrations. This can be explained by considering that as the hydroxide concentration decreased, the number of exposed hard Lewis acid sites on the stationary phase not interacting with an OH- increased and the analytes could interact with them more. Column efficiency (number of plates) decreased as the TMSOH concentration decreased. In the case of 2-amino-3-phenyl-1-propanol, the analyte could not be eluted with the two lowest TMSOH concentrations due to its very strong interactions with the stationary phase.

Table 1 shows the columns efficiencies (number of plates) found for the samples tested. The k' values were 0.58 for tetrapropylammonium chloride, 0.82 for tripropylamine, 1.04 for dipropylamine, 0.38 for butylamine, 0.48 for sec-butylamine and 2.58 for 2-amino-3-phenyl-1-propanol.

Table 1. Column efficiency vs. concentration of TMSOH.

	Column efficiency (number of theoretical plates)				
	31.25 mM	62.5 mM	125 mM	250 mM	500 mM
Tetrapropylammonium chloride	7100	7800	8000	10000	12000
Tripropylamine	7000	7000	9600	13000	15500
Dipropylamine	7000	7300	7500	10700	14500
Butylamine	-----	300	300	500	650
Sec-butylamine	-----	1000	1000	1400	1900
2-amino-3-phenyl-1-propanol	-----	-----	14	20	25

The results indicated that the best results could be expected with the highest TMSOH concentrations. Therefore all further measurements were made at 500 mM TMSOH additive concentrations.

3.2 Analysis of Quaternary Amines

The first quaternary amines used were n-alkyltrimethylammonium ions. Figure 3 shows the RP-HPLC separation of n-hexyltrimethylammonium cation n-octyltrimethylammonium cation, and n-decyltrimethylammonium cation with a mobile phase containing 500 mM TMSOH, 36.5 % methanol and 63.5 % water. The analytes were weakly retained by the stationary phase, because the solubility of the cations in the

mobile phase was very high. Figure 4 shows the relationship between the log k' of the n-alkyltrimethylammonium cations and their carbon number, Cn. This plot is linear, as expected in reversed-phase HPLC when retention is governed by hydrophobic interactions [141-143].

UV transparent symmetric tetralkylammonium cations were also analyzed to see if their retention behavior would be the same as that of the non-symmetric UV transparent quaternary ammonium cations.

Figure 5 shows the chromatogram of tetra-n-butylammonium cation, tetra-n-pentylammonium cation, tetra-n-hexylammonium cation and tetra-n-heptylammonium cation, with a mobile phase of 500 mM TMSOH, 46.9 % methanol and 53.1 % water, at a temperature of 57.5 C. A linear relationship was observed between the log k' and the carbon number, as can be seen in Figure 6. Only cations with long alkyl chains were used because the ones with shorter alkyl chains had too small a retention for the accurate determination of the k' values.

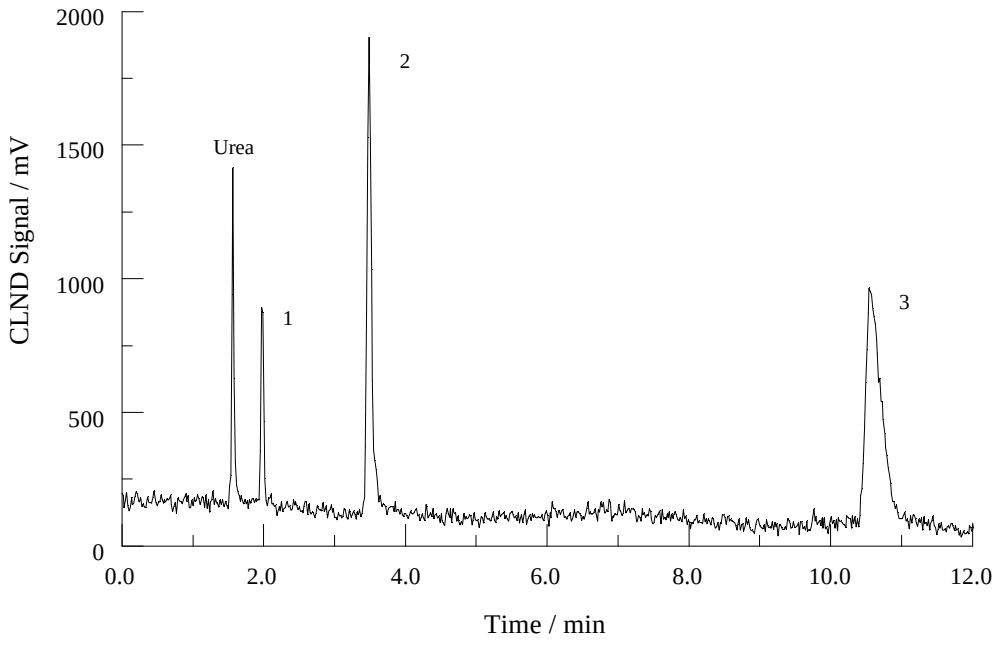


Figure 3. Chromatogram of n-alkyltrimethylammonium cations. Peak 1:n-hexyltrimethylammonium cation, peak 2:n-octyltrimethylammonium cation, peak 3:n-decyltrimethylammonium cation. Mobile phase:500 mM TMSOH, 36.5 % methanol : 63.5 % water. T=50.5 C.

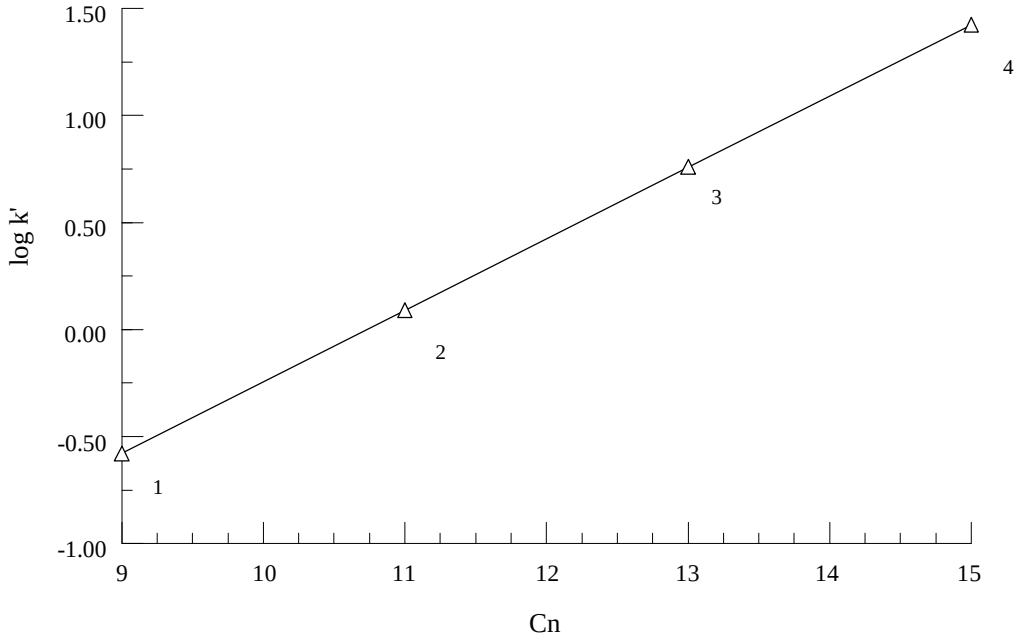


Figure 4. Plot of log k’ vs. Cn for n-alkyltrimethylammonium cations. Point 1:n-hexyltrimethylammonium cation, point 2:n-octyltrimethylammonium cation, point 3:n-decyltrimethylammonium cation, point 4:n-dodecyltrimethylammonium cation. Conditions same as in Figure 2.

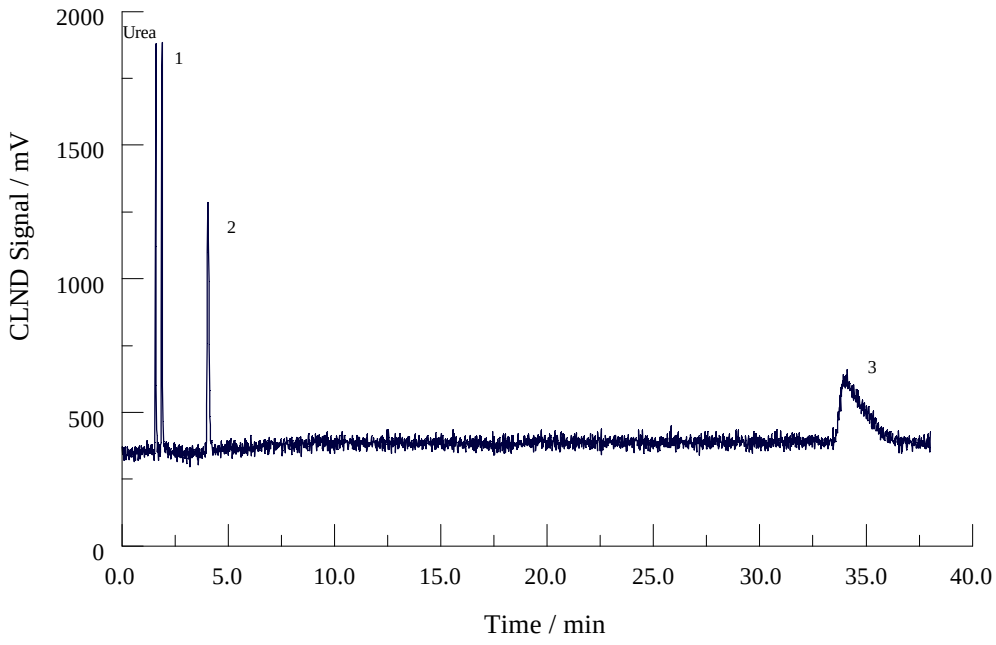


Figure 5. Chromatogram of tetraalkylammonium cations. Peak 1:tetra-n-butylammonium cation, peak 2: tetra-n-pentylammonium cation, peak 3:tetra-n-hexylammonium cation. Mobile phase used:500 mM TMSOH, 46.9 % : 53.1 % water. T= 57.5 C.

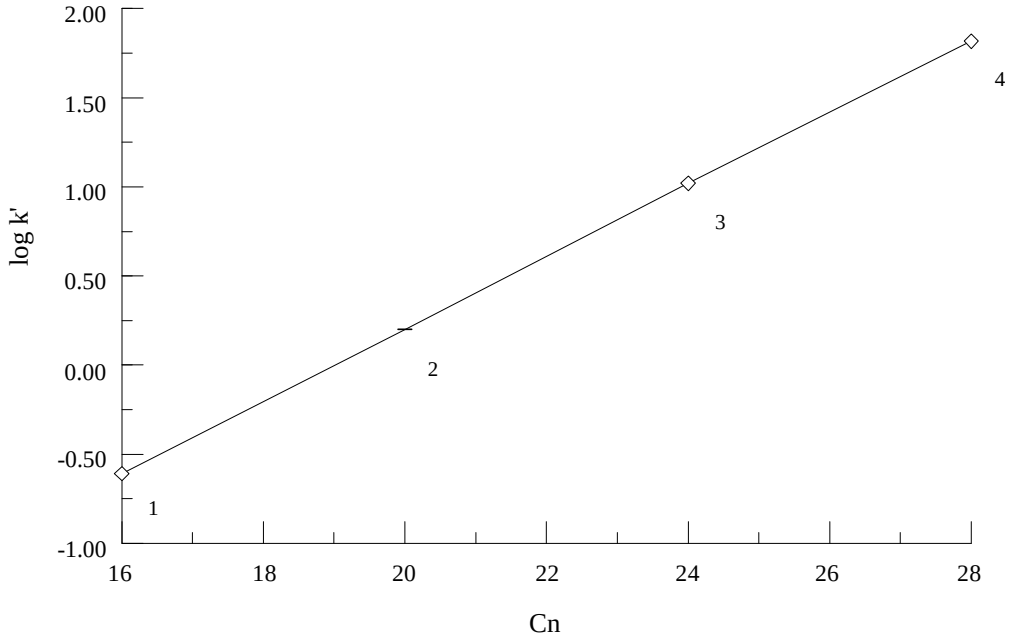


Figure 6. Plot of log k’ vs. Cn for tetraalkylammonium cations. Point 1:tetrabutylammonium cation, point 2:tetrapentylammonium cation, point 3:tetrahexylammonium cation, point 4:tetraheptylammonium cation. Conditions same as in Figure 4.

After these UV transparent quaternary amines were separated, UV absorbing quaternary amines such as benzyltrialkylammonium cations, were analyzed to find out if they gave similar results.

Figure 7 shows the chromatogram where benzyltrimethyl-, benzyltriethyl-, benzyltripropyl- and benzyltributylammonium cations are separated. The retention of these analytes is a little higher than the retention of the corresponding tetraalkylammonium cations. The peak shape observed for all of them was symmetric.

The log k' vs. Cn relationship shown in Figure 8 is not linear. The aromatic group of the molecule is large enough to interact with the stationary phase, however, in the case of the benzyltrimethyl-, benzyltriethyl- and benzyltripropylammonium cations, the alkyl groups are so short that they may not be able to interact with the stationary phase at the same time that the aromatic ring does, therefore leading to weaker than expected hydrophobic interactions with the stationary phase.

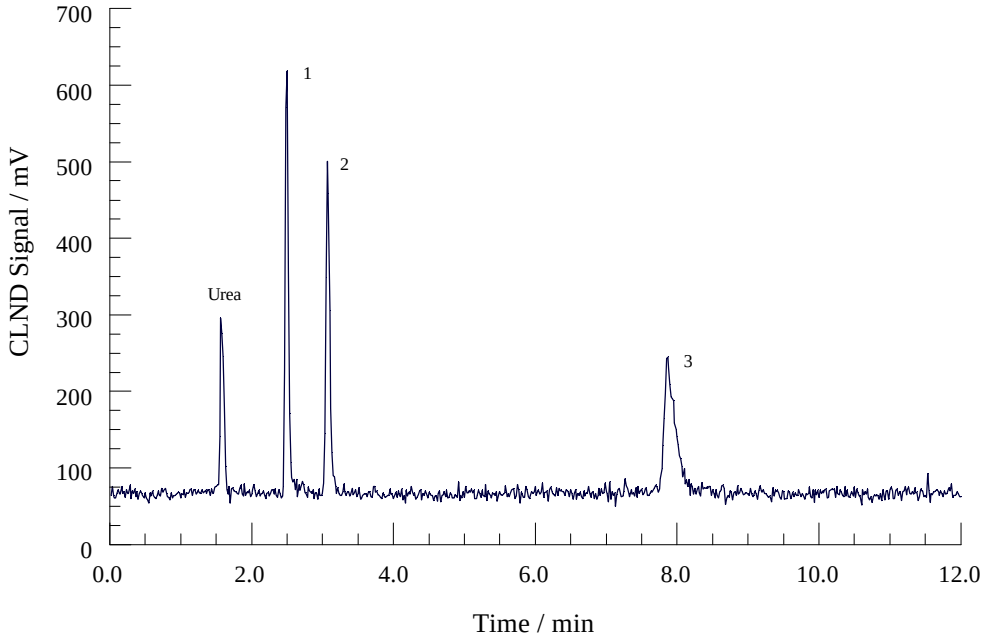


Figure 7. Chromatogram of benzyltrialkylammonium cations. Peak 1:benzyltrimethylammonium cation, peak 2:benzyltriethylammonium cation, peak 3:benzyltripropylammonium cation, peak 4: benzyltributylammonium cation. Mobile phase:500 mM TMSOH, 5.1 % methanol and 94.9 % water. T=50.5 C.

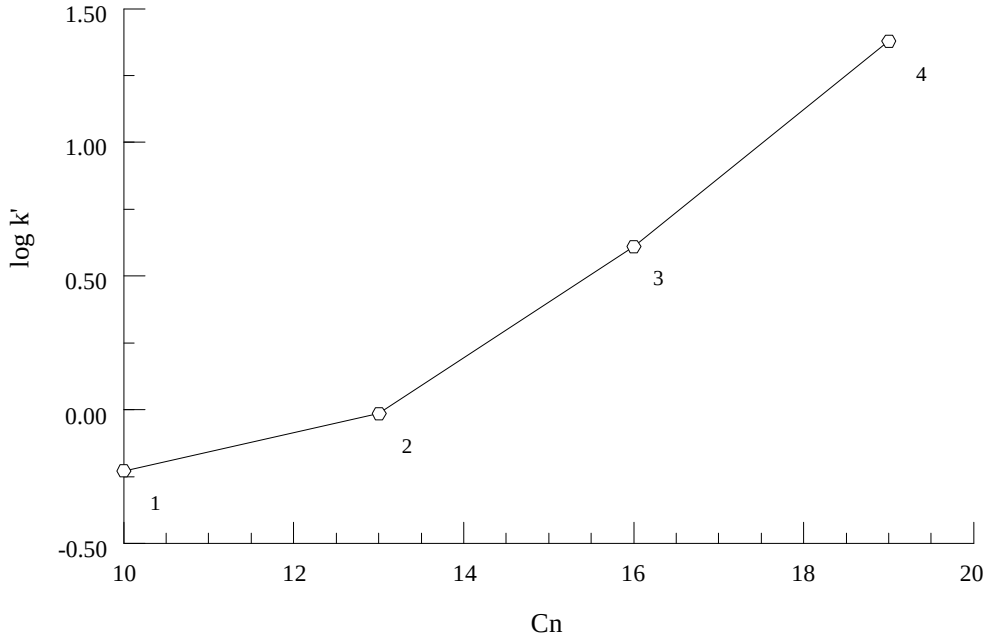


Figure 8. Plot of log k’ vs. Cn for benzyltrialkylammonium cations. Point 1:benzyltrimethylammonium cation, point 2:benzyltriethylammonium cation, point 3:benzyltripropylammonium cation, point 4: benzyltributylammonium cation. Conditions used are the same as in Figure 6.

3.3 Analysis of Tertiary Amines

After the quaternary amines, some tertiary amines were analyzed. Dimethylethylamine, dimethylbutylamine and dimethylhexylamine were analyzed using a mobile phase containing 500 mM TMSOH, 2 % MeOH and 98 % water. The chromatogram for their separation is shown in Figure 9. All three amines were weakly retained and the peak shape observed for all of them was symmetric. A linear relationship was observed between the log k' and their carbon number (Figure 10). The analytes can indeed be separated as neutral compounds and the system used behaves as a reversed-phase HPLC system as expected.

After looking at the dimethylalkylamines, there was interest in analyzing symmetric UV transparent tertiary amines to see if they would have similar interactions with the stationary phase. Figure 11 shows the chromatogram for the separation of tri-n-propylamine, tri-n-butylamine and tri-n-pentylamine using a 500 mM TMSOH, 57.3 % methanol and 42.7 % water mobile phase at 60 C. The retention of the analytes is higher than for quaternary ammonium cations of similar carbon number, as expected, due to the absence of the positive charge. Figure 12 shows the linearity of the plot of log k' vs. Cn for the same analytes.

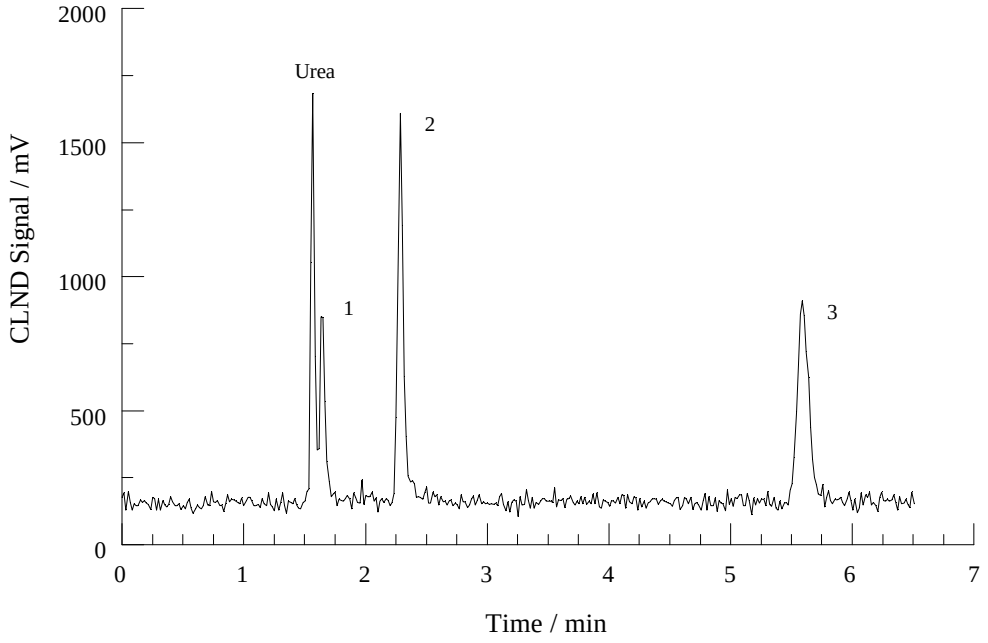


Figure 9. Chromatogram of dimethylalkylamines. Peak 1:dimethylethylamine, peak 2:dimethylbutylamine, peak 3:dimethylhexylamine. Mobile phase:500mM TMSOH, 2 % methanol : 98 % water. T=50.5 C.

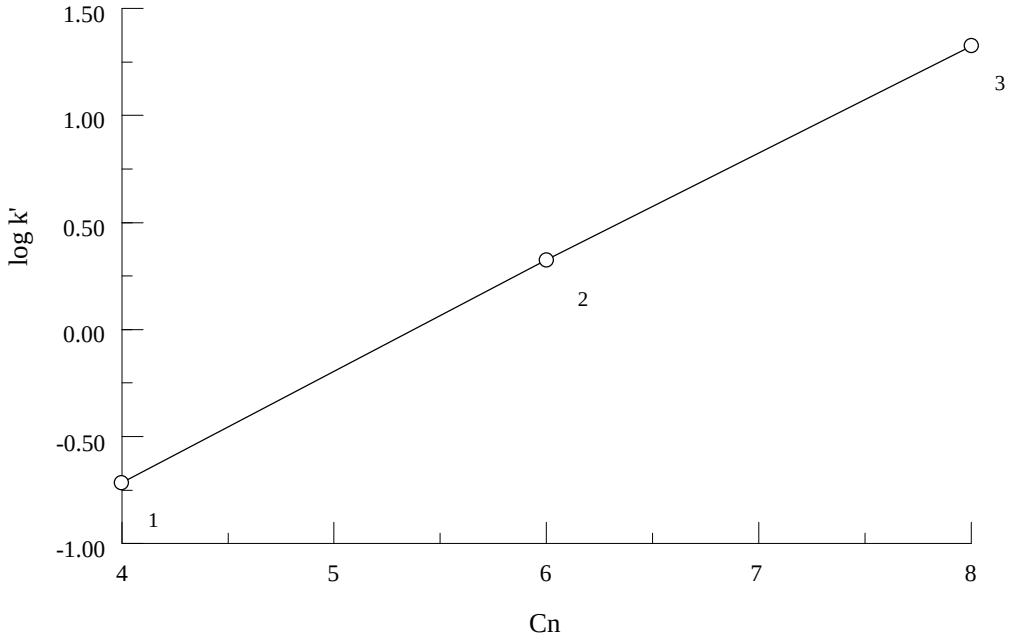


Figure 10. Plot of log k’ vs. Cn for dimethylalkylamines. Point 1:dimethylethylamine, point 2: dimethylpropylamine, point 3:dimethylbutylamine. Conditions same as in Figure 9.

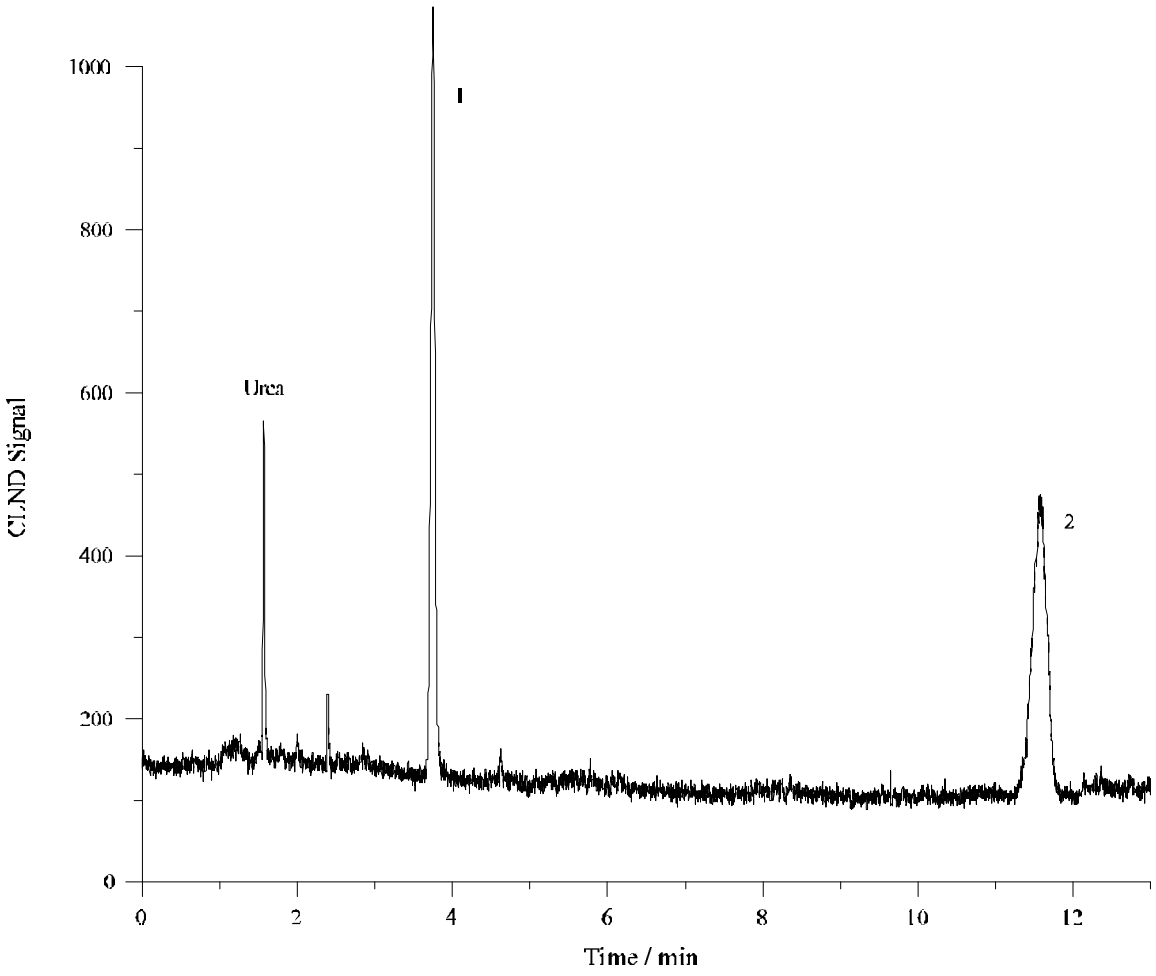


Figure 11. Chromatogram of trialkylamines. Peak 1:tripropylamine, peak 2:tributylamine. Mobile phase:500 mM TMSOH, 57.3 % methanol : 42.7 % water. T=60.0 C.

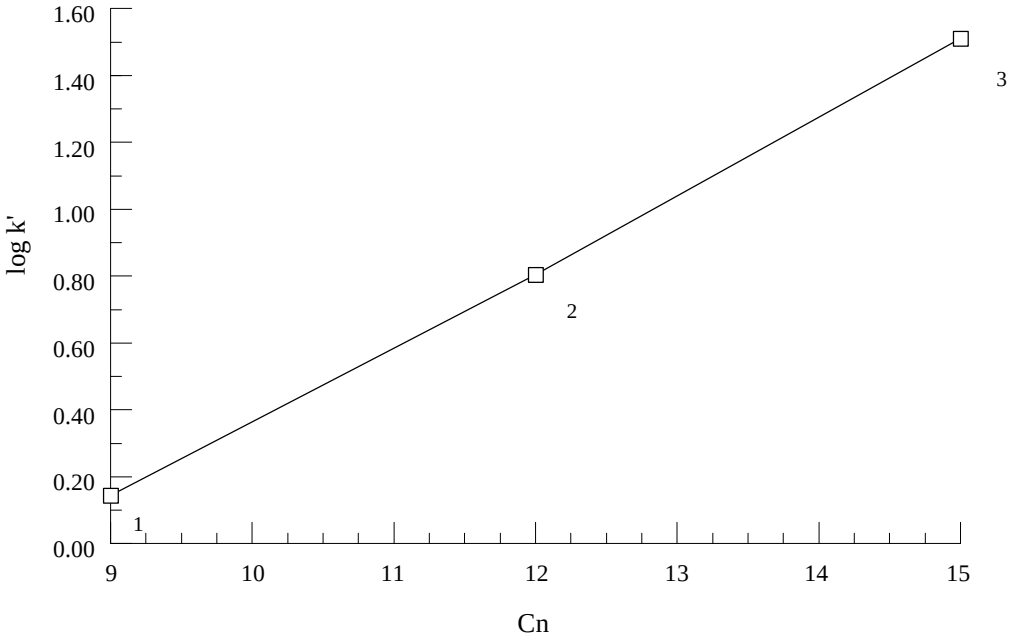


Figure 12. Plot of log k’ vs. Cn for trialkylamines. Point 1:tripropylamine, point 2:tributylamine, point 3: tripentylamine. Conditions same as in Figure 11.

3.4 Analysis of Secondary Amines

In the same way as quaternary and tertiary amines were analyzed, secondary amines were also studied. First, secondary amines containing an aromatic group were analyzed. N-methylbenzylamine, N-ethylbenzylamine and N-butylbenzylamine were separated using a mobile phase containing 500 mM TMSOH, 5.1 % methanol and 94.9 % water. The temperature used was 51 C. Not all the peaks observed in the chromatogram (Figure 13) were symmetric. N-methylbenzylamine and N-ethylbenzylamine show a small tail, while N-butylbenzylamine does not. The tail could be caused by the short alkyl chain of N-methyl and N-ethylbenzylamine that can not hinder the interaction of the NH group with the exposed Lewis acid sites of the zirconia-based stationary phase, while N-butylbenzylamine has a longer alkyl chain and might be able to disrupt these interactions.

UV transparent symmetric secondary amines were also tested. Di-n-propylamine, di-n-butylamine and di-n-pentylamine were separated using a mobile phase with 500 mM TMSOH, 5.1 % methanol and 94.9 % water. The temperature used in these experiments was 51 C. As can be seen from the chromatogram in Figure 14, all peaks were symmetric. These analytes were highly retained on the stationary phase and their linear log k' vs. carbon number relationship is shown in Figure 15, indicating that the interactions between the analytes and the stationary phase are mainly hydrophobic.

Di-n-methylamine and di-n-ethylamine were also tested. Figure 16 shows the chromatogram indicating that di-n-ethylamine had a smaller retention than di-n-methylamine. Also, the later showed a tailed peak while the former showed a symmetric peak. The reason for these could be that the alkyl groups in di-n-methylamine are so

small that the secondary amine can interact more freely with the exposed Lewis acid sites of the stationary phase. In the case of the di-n-ethylamine however, the ethyl groups are large enough to interrupt the interaction between the secondary amino group and the Lewis acid sites of the stationary phase, thus the retention is lower and the peak shape is symmetric.

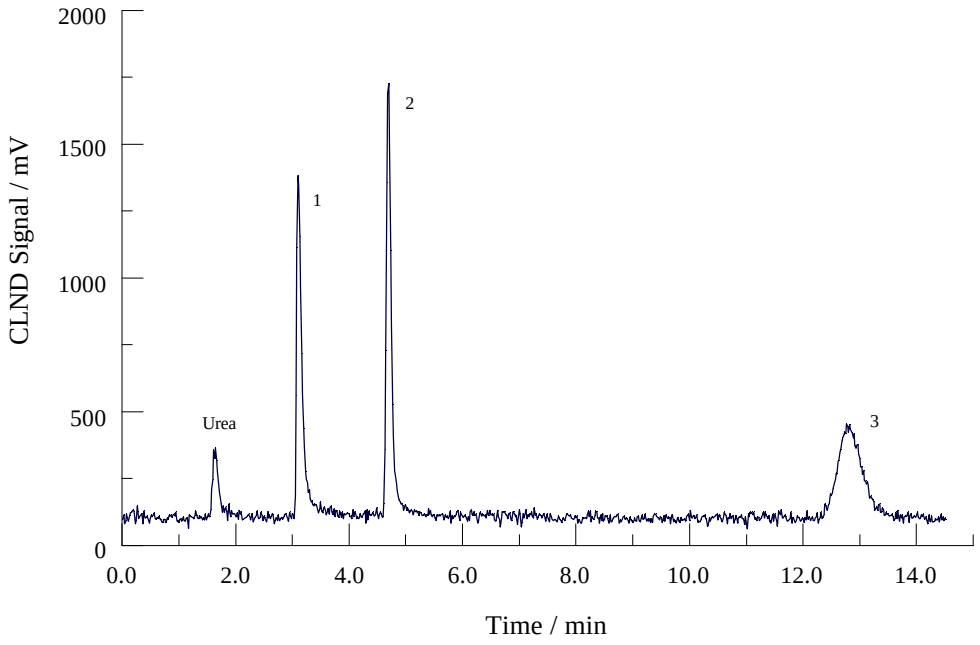


Figure 13. Chromatogram of N-alkylbenzylamines. Peak 1:N-methylbenzylamine, peak 1:N-ethylbenzylamine, peak 3:N-butylbenzylamine. Mobile phase:500 mM TMSOH, 5.1 % methanol : 94.9 % water. T=51 C.

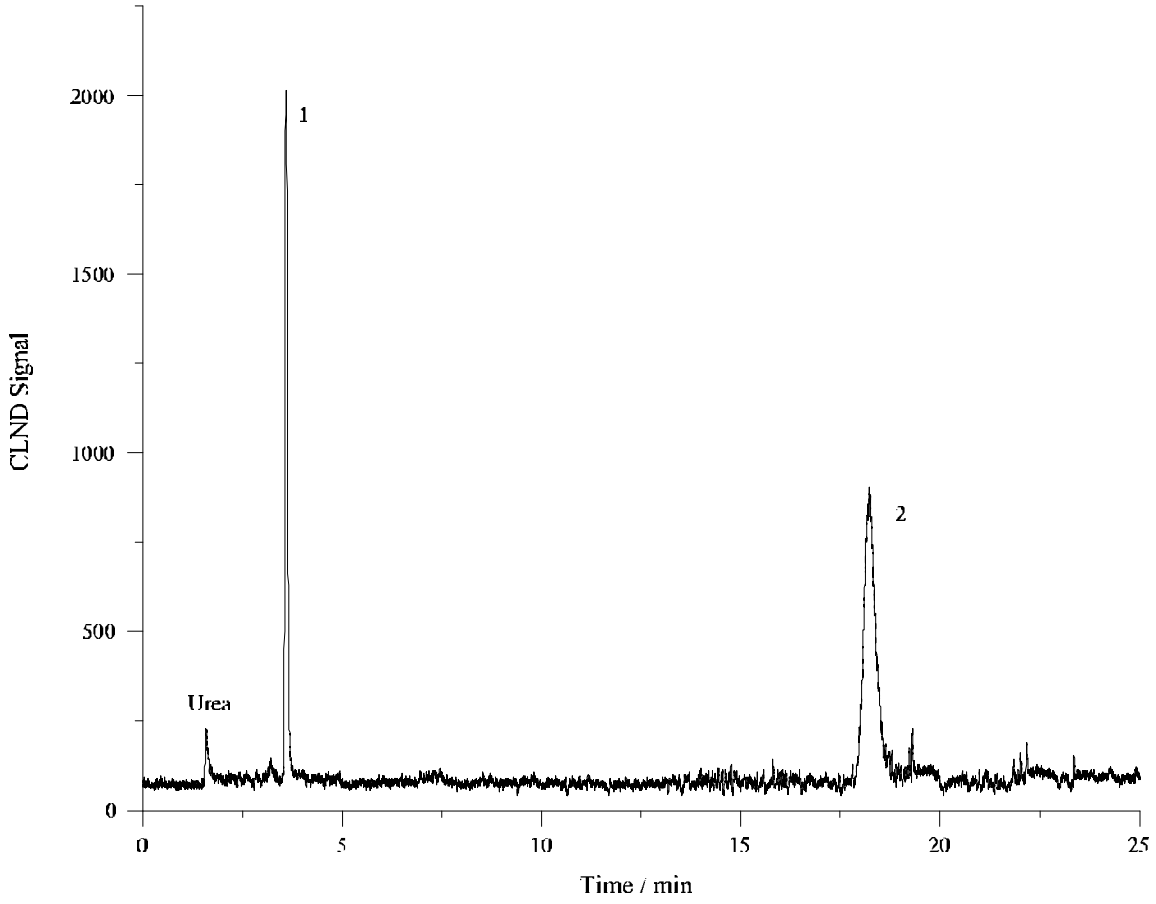


Figure 14. Chromatogram of di-n-alkylamines. Peak 1:di-n-propylamine, peak 2:di-n-butylamine. Mobile phase:500 mM TMSOH, 5.1 % methanol : 94.9 % water. T=51 C.

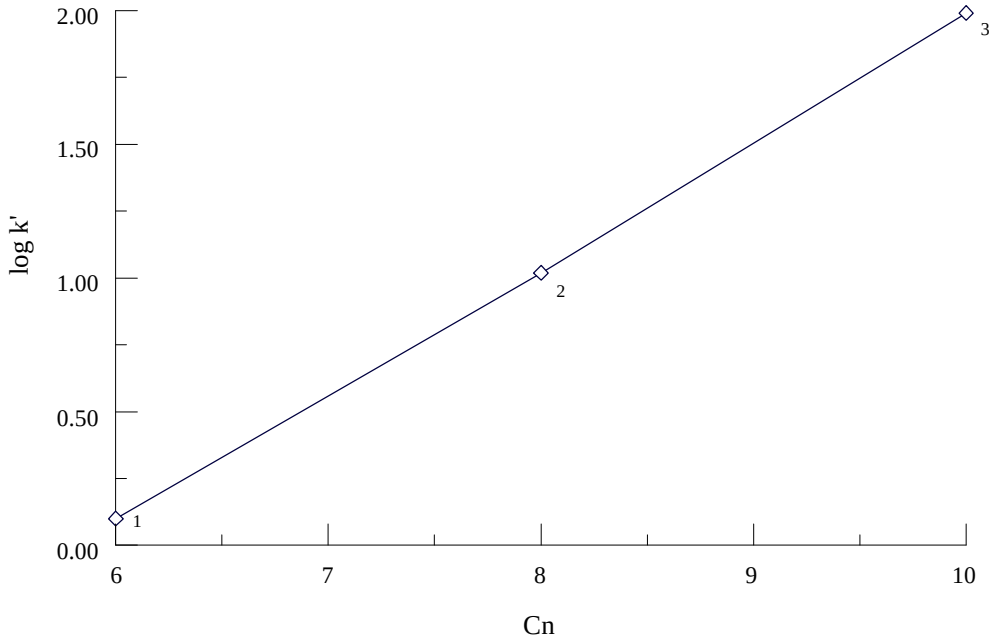


Figure 15. Plot of log k’ vs. Cn for di-n-alkylamines. Peak 1:di-n-propylamine, peak 2:di-n-butylamine, peak 3:di-n-pentylamine. Conditions same as in Figure 14.

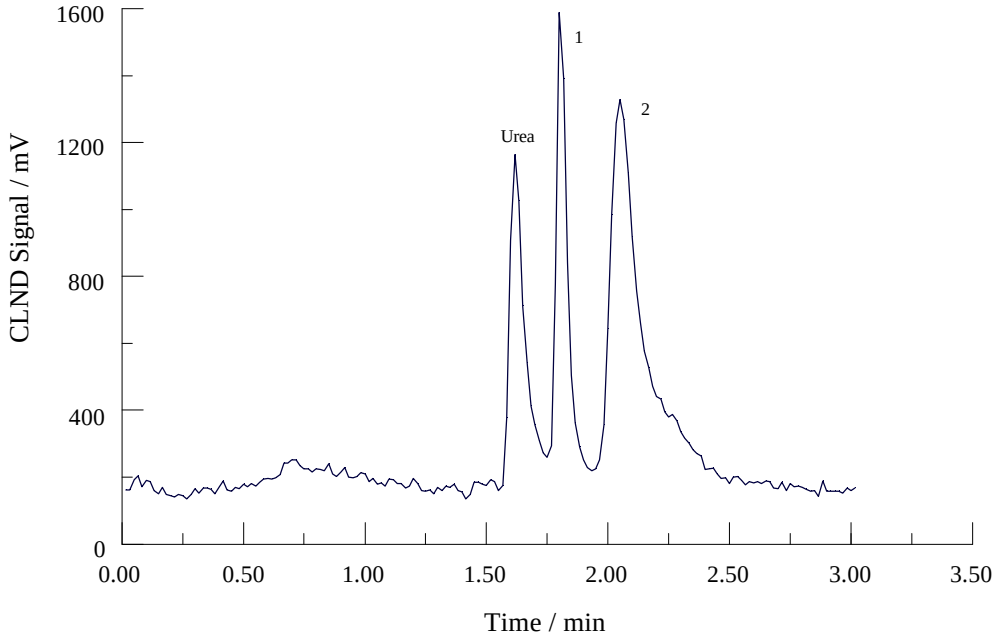


Figure 16. Chromatogram of diethylamine (peak 1) and dimethylamine (peak 2). Conditions same as in Figure 15.

3.5 Analysis of Primary Amines

The last type of amino group to study was the primary amino group. Figure 17 shows a chromatogram for the separation of n-butylamine, n-hexylamine and n-octylamine. The mobile phase used in that experiment contained 500 mM TMSOH, 36.5 % methanol and 63.5 % water. The peaks tail strongly compared to the previous samples analyzed. Though the zirconia particles are covered with a polybutadiene polymer, the polymer may have some open patches where the zirconia surface is exposed. These exposed Lewis acid sites could easily interact with the hard Lewis base amino group at the end of the alkyl chain.

To test this hypothesis, compounds containing the primary amino group in the middle of the alkyl chain have been analyzed. Figure 18 shows the chromatograms of n-pentylamine, 2-aminopentane and 3-aminopentane. All analytes were run separately using mobile phases with different methanol concentrations in order to adjust their k' to similar values to permit comparison of their peak widths. The longest peak tail was observed for n-pentylamine, with a peak asymmetry (A_s) of 6.6, followed by 2-aminopentane (A_s = 3.1) and then 3-aminopentane (A_s = 2.13). The plate number found for n-pentylamine was 1100, for 2-aminopentane it was 4000 and for 3-aminopentane it was 6800 indicating that as the amino group was moved into the middle of the alkyl chain, the interactions of the amino group with the Lewis acid sites of the zirconia-based stationary phase may have been diminished due to steric hindrance. However, the A_s values shows that there is still some interaction between the analyte and the stationary pahse since the As values found for all of them are higher than 1.5.

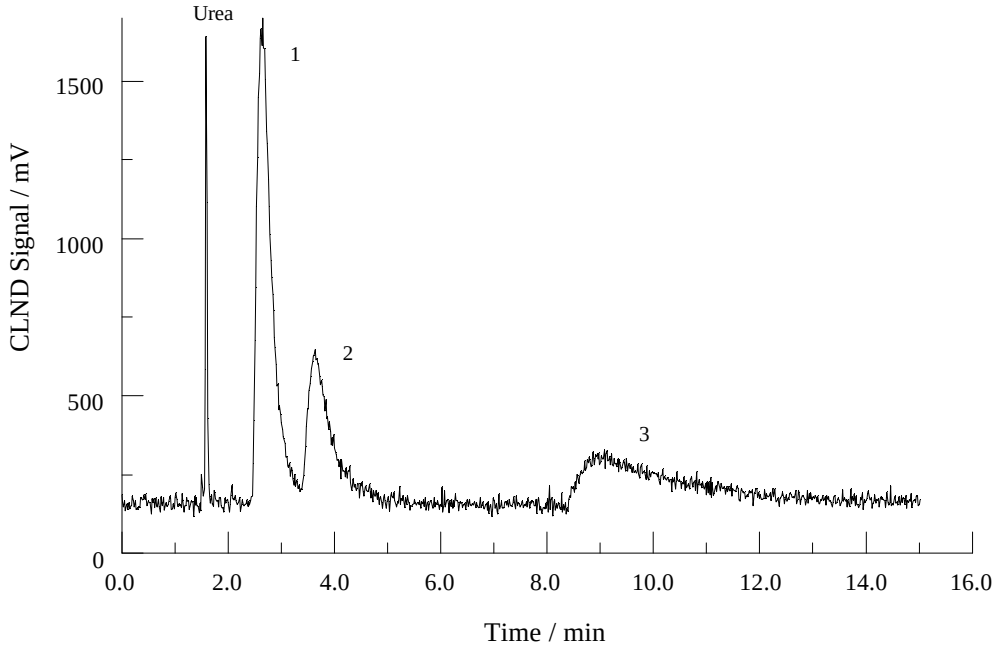


Figure 17. Chromatogram of n-alkylamines. Peak 1:n-butylamine, peak 2:n-hexylamine, peak 3:n-octylamine. Mobile phase:500 mM TMSOH, 36.5 % methanol : 63.5 % water. T=50.5 C.

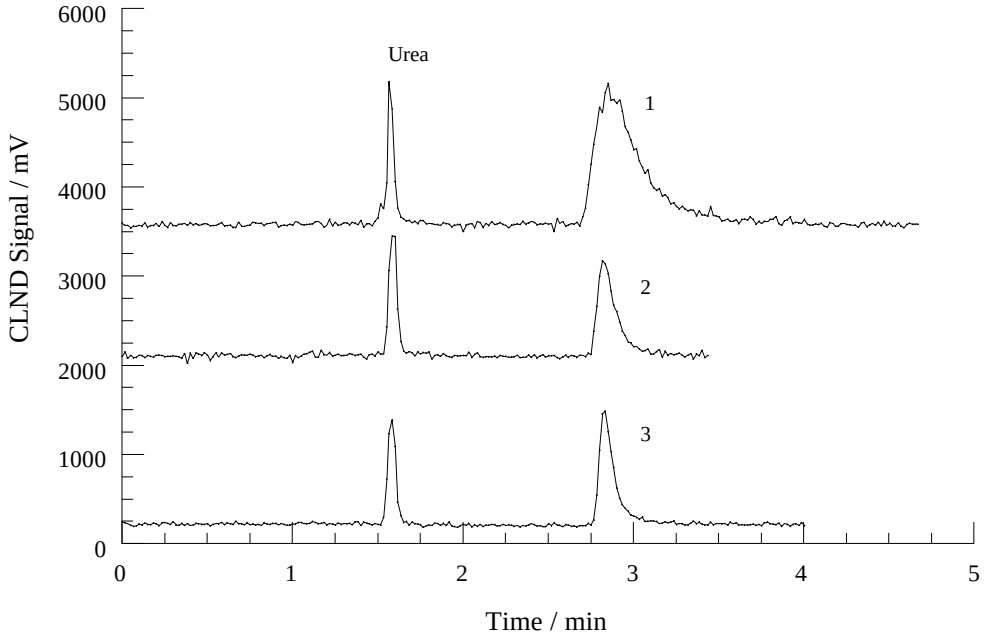


Figure 18. Chromatograms of isomers of pentylamine. Peak 1:n-pentylamine, mobile phase:500 mM TMSOH, 36.5 % methanol : 63.5 % water. Peak 2:2-aminopentane, mobile phase:500 mM TMSOH, 6.9 % methanol:94.1 % water. Peak 3:3-aminopentane. Mobile phase:500 mM TMSOH, 2 % methanol : 98 % water. T=50.5 C.

To further corroborate this hypothesis, isomers of heptylamine were also analyzed. Chromatograms of n-heptylamine, 2-aminoheptane, 3-aminoheptane and 4-aminoheptane are shown in Figure 19. The same trend was observed as for the isomers of pentylamine. The peak tail is the longest for n-heptylamine (A_s = 6.6), followed by 2-aminoheptane (A_s = 3.625). The plate number for n-heptylamine was 2500, while the plate number for 2-aminoheptane was 6600. The peak shape for 3-aminoheptane (A_s = 1.875) also shows a drastic improvement, corroborated by the respective plate number of 11500. However, there is not much improvement between the 3- and the 4-aminoheptane (A_s = 1.625) as the peak tail for both of them is almost identical. The plate number for 4-aminoheptane is 12000 indicating a peak shape improvement of only about 5 % with respect to the 3-aminoheptane.

If these values obtained for peak asymmetry are compared to the values obtained for n-pentylamine, both n-pentyl and n-heptylamine have the same values for peak asymmetry. However, for the isomers of pentylamine the lowest value of A_s found was 2.13, for 3-pentylamine. For the isomers of heptylamine, a value of 1.625 is obtained for 4-heptylamine, which corroborates the prediction that the alkyl chain hinders the interaction between the amino group and the Lewis acid sites exposed on the PBD-zirconia column.

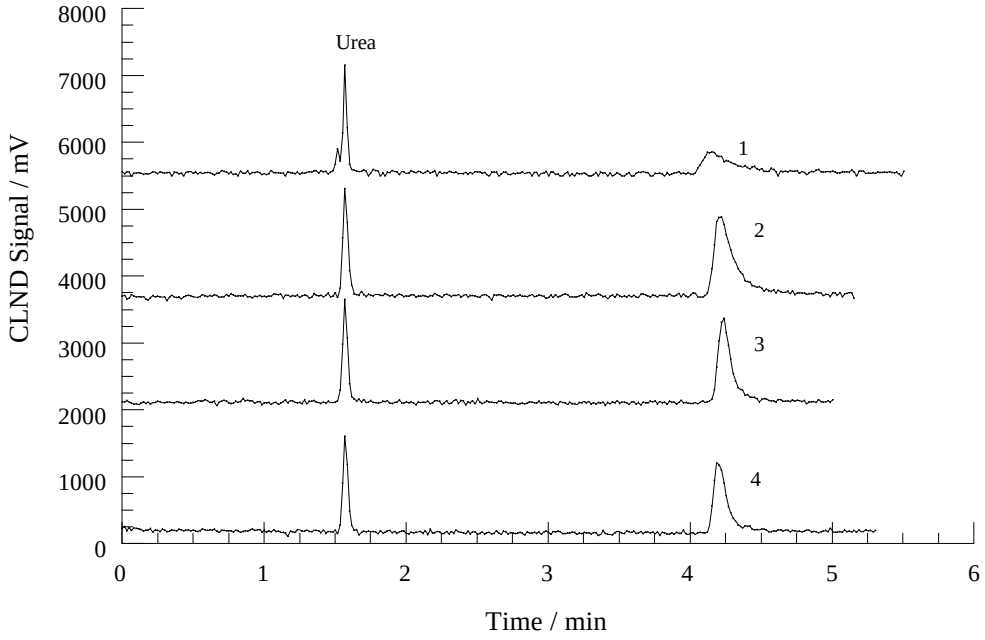
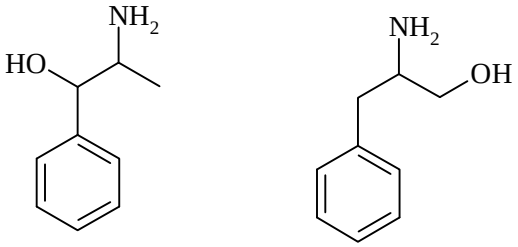


Figure 19. Chromatograms of isomers of heptylamine. Peak 1:n-heptylamine, mobile phase:500 mM TMSOH, 36.5 % methanol : 63.5 % water. Peak 2:2-aminoheptane, mobile phase:500 mM TMSOH, 28 % methanol : 72 % water. Peak 3:3-aminoheptane, mobile phase:500 mM TMSOH, 25 % methanol : 75 % water. Peak 4:4-aminoheptane, mobile phase:500 mM TMSOH, 25.5 % methanol, 74.5 % water. T=50.5 C.

3.6 Analysis of Amines That Contain an Alcohol Group

After the simple amines were analyzed, it was important to look at the analysis of compounds containing both an alcohol group and an amino group.

First, norephedrine (2-amino-1-phenyl-1-propanol) and 2-amino-3-phenyl-1-propanol shown in Figure 20 were analyzed.



norephedrine 2-amino-3-phenyl-1-propanol

Figure 20. Structures of norephedrine and 2-amino-3-phenyl-1-propanol.

Figure 21 shows the chromatograms of norephedrine and 2-amino-3-phenyl-1-propanol, both analytes show a long tail. Then, amphetamine was analyzed and its chromatogram was compared to that of norephedrine. Figure 22 shows the structure of amphetamine. Figure 23 shows the chromatograms of norephedrine and amphetamine, amphetamine shows a symmetric peak. After this, ephedrine and methamphetamine were analyzed, their structures are shown in Figure 24, their chromatograms in Figure 25.

The last sample analyzed was N-methylephedrine. Its structure is shown in Figure 26. Figure 27 shows chromatograms for all three, norephedrine, ephedrine and N-

methylephedrine. Norephedrine showed the longest tail, followed by ephedrine. N-methylephedrine showed a symmetric peak.

The peak tailing observed for norephedrine and 2-amino-3-phenyl-1-propanol could be caused by interactions between the amino group and the hard Lewis acid sites on the zirconia surface. However, both amino groups are somewhat hindered (2-amino groups) and tailing is worse than what was observed for 2-amino alkanes. (see section 3.4) or amphetamine (2-amino-3-phenyl-propane) as shown in Figure 22.

As can be seen from Figure 21, 2-amino-3-phenyl-1-propanol has a much longer tail than norephedrine. Both compounds have their amino groups somewhat hindered. However, amphetamine also has its amino group hindered to the same extent, or even less, yet it does not show a peak tail.

Since an alcohol group is also present in norephedrine and 2-amino-3-phenyl-1-propanol, it is possible that it also interacts with the Lewis acid sites of the zirconia surface, just as the primary amino group does, because the alcohol group is also a hard Lewis base.

The alcohol group is hindered in norephedrine, but not in 2-amino-3-phenyl-1-propanol. In fact, the alcohol group in the later is much more accessible for interaction with Lewis acid sites. It can be hypothesized that both the amino and the alcohol groups interact at the same time with the exposed hard Lewis acid sites of the zirconia surface forming a bridge. The reason why such a severe tail is observed for 2-amino-3-phenyl-1-propanol is because there is a small number of sites available located at appropriate

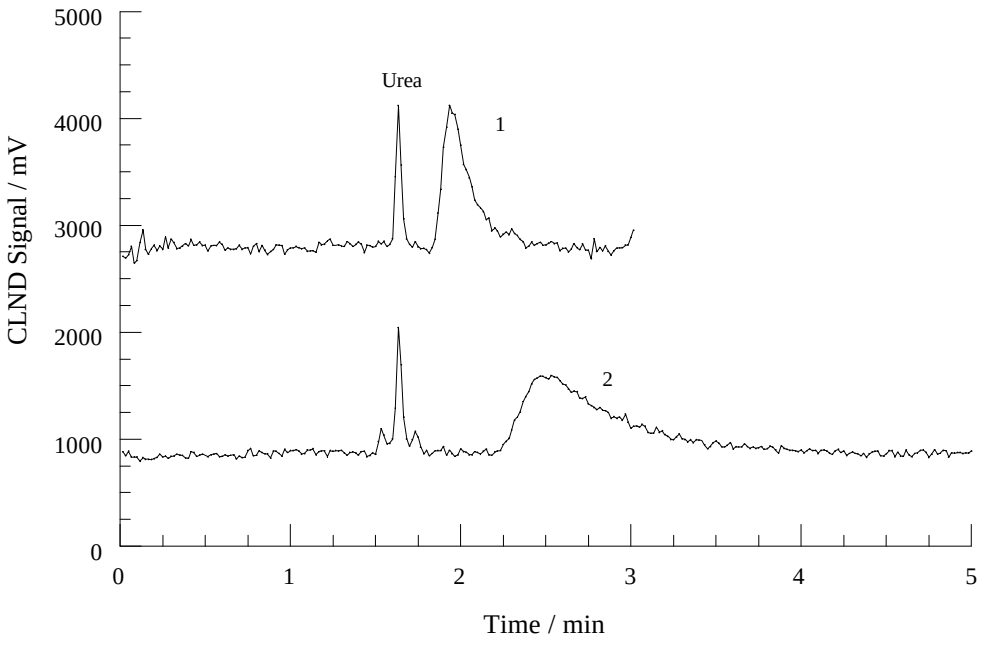


Figure 21. Chromatograms of norephedrine (peak 1) and 2-amino-3-phenyl-1-propanol (peak 2). Mobile phase:500 mM TMSOH, 36.5 % methanol : 63.5 % water. T=50.5 C.

distances from each other for simultaneous interactions, slowing only some molecules in the band down while the rest of the molecules are moved by the mobile phase.

2-Amino-3-phenyl-1-propanol has a higher possibility of finding two exposed Lewis acid sites than norephedrine, therefore it has a longer tail.

To further test this hypothesis, amphetamine was also analyzed.

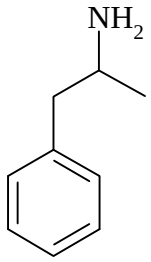


Figure 22. Structure of amphetamine.

Amphetamine has the same structure as norephedrine with the exception that it does not have an alcohol group in its structure. Amphetamine therefore can only have a one-site interaction with the Lewis acid sites of the stationary phase and since the primary amino group is hindered, the peak tail should be small, as it is shown in Figure 23. The plate number observed for norephedrine was 1000, while the plate number observed for amphetamine was 9000.

In order to explain the observed tailing behavior in greater detail, norephedrine was analyzed using capillary electrophoresis. The background electrolyte used was the HPLC mobile phase that contained 500 mM TMSOH, 5.1 % methanol and 94.9 % water. This was done to find out whether the alcohol group present in the analyte was

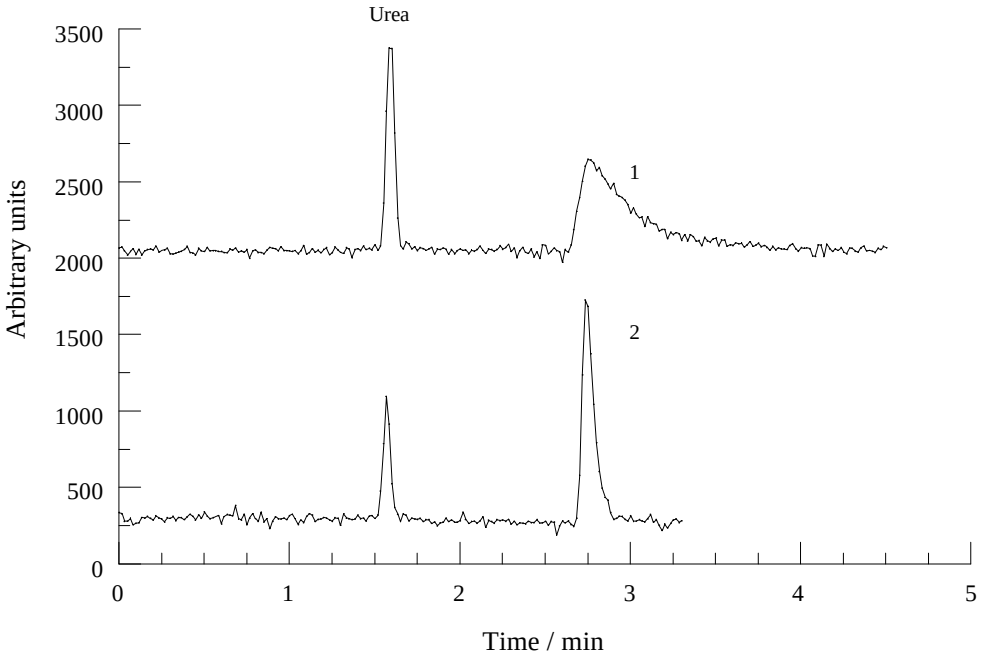
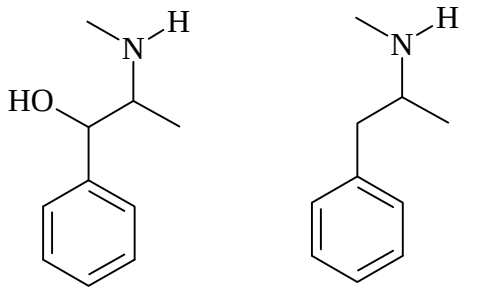


Figure 23. Chromatograms of norephedrine and amphetamine. Peak 1:norephedrine, mobile phase:500 mM TMSOH, 2 % methanol : 98 % water. Peak 2:amphetamine, mobile phase:500 mM TMSOH, 25.5 % methanol : 74.5 % water. T=50.5 C.

dissociated under the conditions the HPLC separations were run. The results obtained indicate that norephedrine had an effective mobility of -2.26×10^{-5} cm^2/Vs. The anionic mobility means that the OH group had to be dissociated. This corroborates the prediction introduced earlier about the bridging interactions of the analyte through both its amino and alkoxide functional groups and the Lewis acid sites of the zirconia surface.

Ephedrine was also analyzed and its retention behavior was compared to that of methamphetamine. Ephedrine and methamphetamine have the same structure except for the absence of the alcohol group in methamphetamine, Figure 24. In Figure 25, both analytes have the same retention, yet ephedrine tails more than methamphetamine.



ephedrine methamphetamine

Figure 24. Structures of ephedrine and methamphetamine.

These two structures have a secondary amino group, which does not cause as big a peak tail as primary amines do. Ephedrine shows a tail, albeit small, because the methyl group present on the amino group disrupts some of the bridge formation, depending on the orientation of the amino group with respect to the stationary phase.

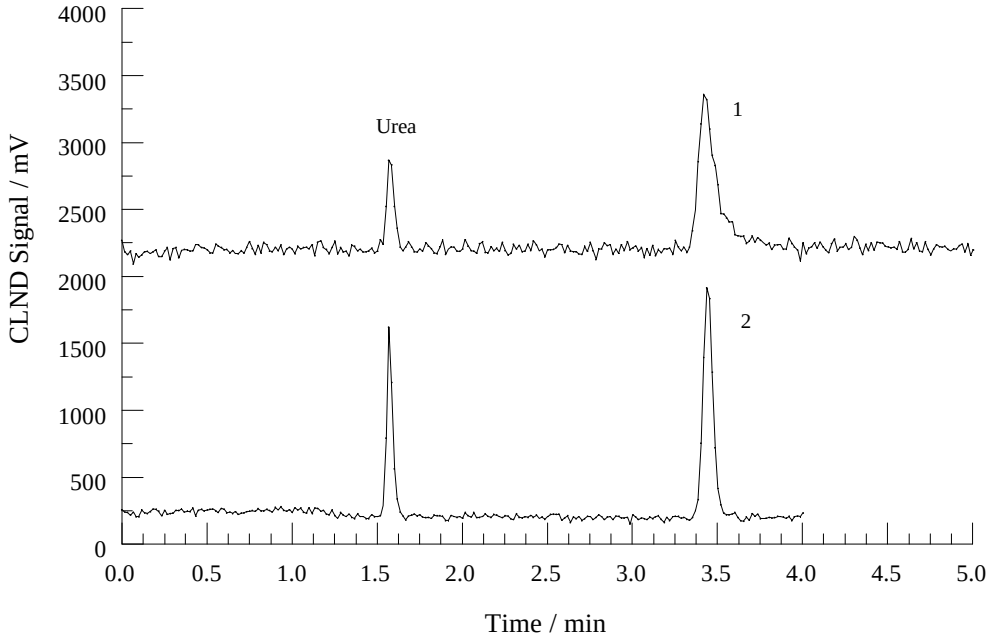


Figure 25. Chromatograms of ephedrine and methamphetamine. Peak 1:ephedrine, mobile phase:500 mM TMSOH, 5.1 % methanol : 94.9 % water. Peak 2:methamphetamine, mobile phase:500 mM TMSOH, 25.5 % methanol : 74.5 % water. T=50.5 C.

Therefore, the peak tail of ephedrine should be smaller than that of norephedrine, as bridge formation is less favored. In the case of methamphetamine, there is no alcohol group present, therefore no bridge formation can occur. The plate number observed for ephedrine was 5500, the plate number observed for methamphetamine was 17000.

Capillary electrophoresis was also used to determine if the OH group in ephedrine was dissociated or not under the conditions of the reversed-phase HPLC separation. Again, the HPLC mobile phase was used as the background electrolyte in capillary electrophoresis. It was found that ephedrine had an effective mobility of -1.91×10^{-5} cm^2/Vs, indicating the presence of a negatively charged alkoxide group and offering the possibility of interaction with the Lewis acid sites of zirconia.

In the last experiment, the peak shape of N-methylephedrine (structure shown in Figure 26) was compared with that of the primary and secondary amine analogs, norephedrine and ephedrine, respectively.

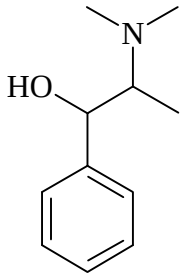


Figure 26. Structure of N-methylephedrine.

Figure 27 shows chromatograms comparing the peak shapes of norephedrine, ephedrine and N-methylephedrine. All analytes have the same structure with the exception of the amino group, which changes from primary through secondary to tertiary, respectively. The primary amino group leads to the longest tail followed by the secondary and then the tertiary amino group.

Capillary electrophoresis was also used to determine whether the OH group in N-methylephedrine was dissociated or not under the conditions of the HPLC separation. N-methylephedrine had an effective mobility of -1.84×10^{-5} cm^2/Vs, corroborating the presence of a negatively charged alkoxide group. Yet the peak shape observed was symmetric and the plate number found for the analyte was 15000. This is because the peak tail is aggravated only when a bridge is formed.

3.7 Analysis of Pharmaceuticals

After studying the retention and peak shape of amines, some common drugs and their intermediates were separated. The analytes have been divided into groups according to similarities in their structures. Figure 28 shows the group of analytes that contain only amino groups in their structures.

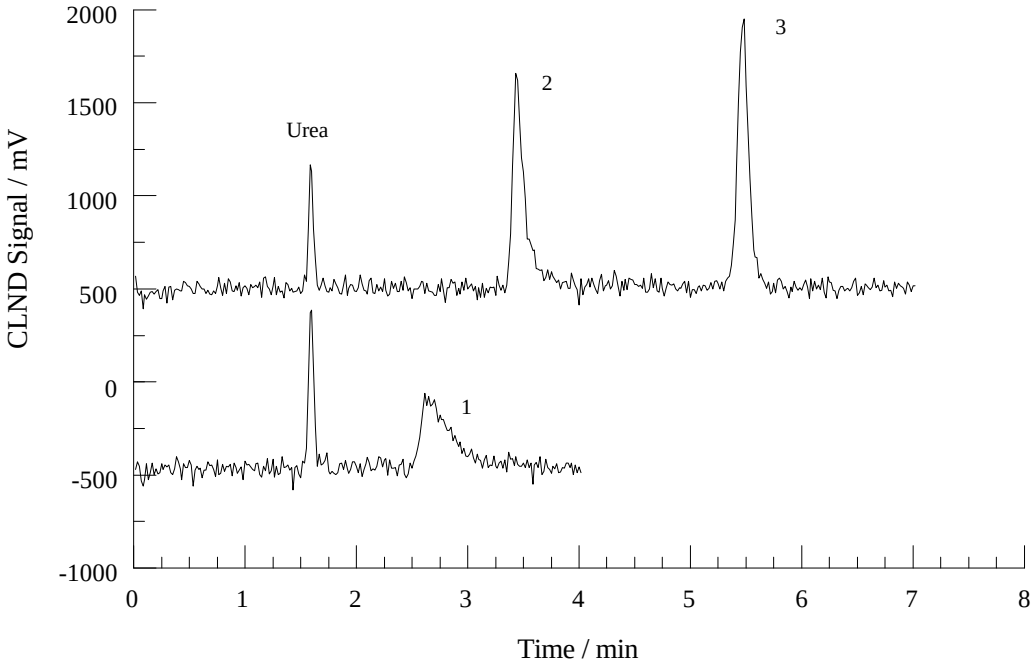
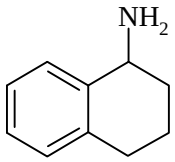
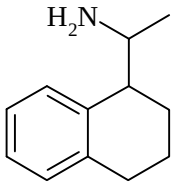


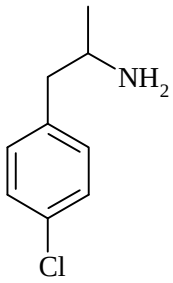
Figure 27. Chromatograms of norephedrine, ephedrine and N-methylephedrine. Peak 1:norephedrine, peak 2:ephedrine, peak 3:N-methylephedrine. Mobile phase:500 mM TMSOH, 5 % methanol : 95 % water. T=50.5 C.



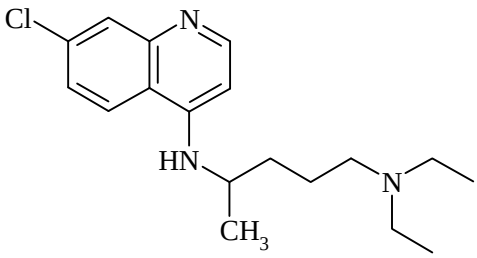
28-1 : 1,2,3,4-tetrahydro-naphthylamine



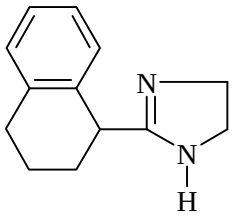
28-2 : 1-naphthyl-1-ethylamine



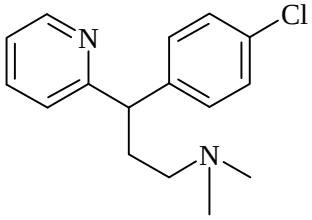
28-3 : 4-chloramphetamine



28-4 : chloroquine



28-5 : tetrahydrozoline



28-6 : chlorpheniramine

Figure 28. Structures of analytes containing only amino groups.

Figure 29 shows a group of analytes with structures containing ether groups as well as secondary or tertiary amine groups.

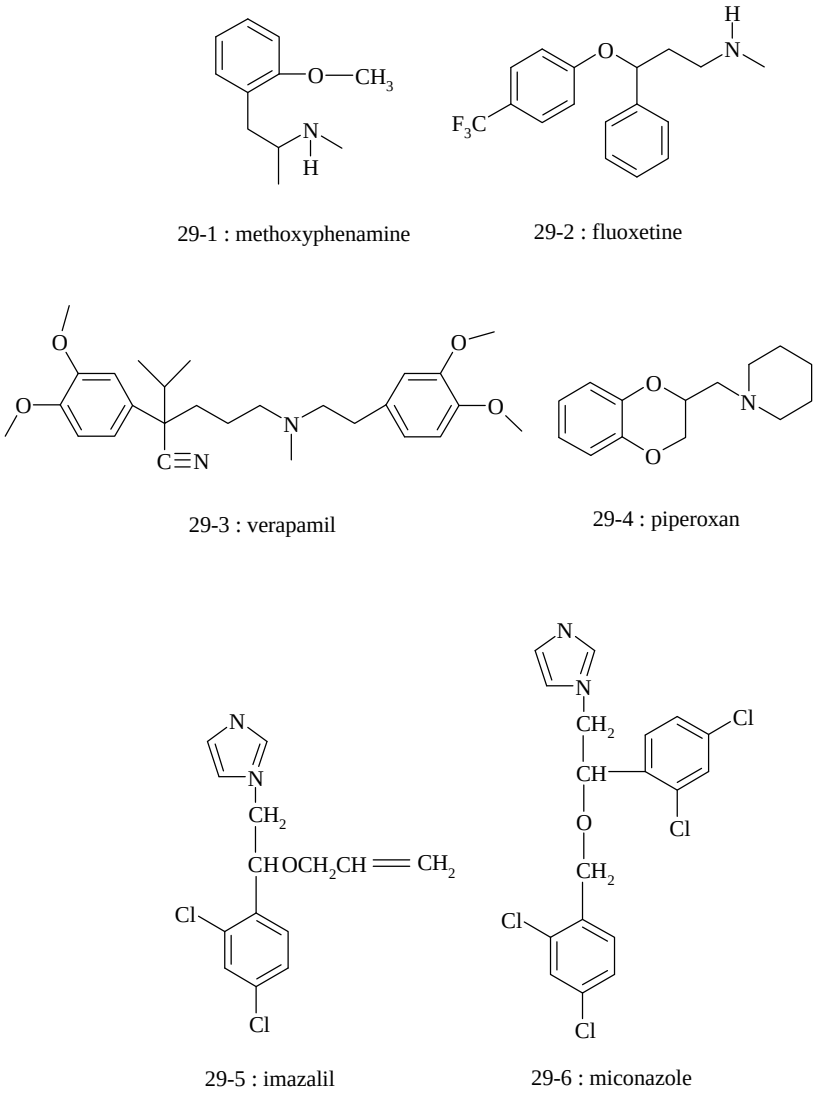
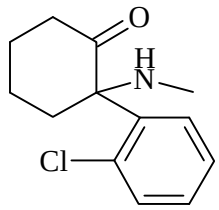
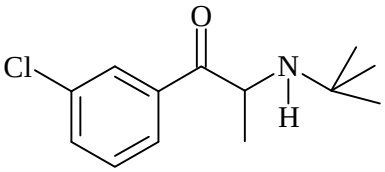


Figure 29. Structures of analytes containing ether groups and secondary or tertiary amino groups.

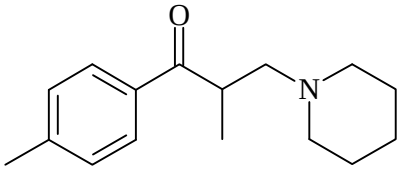
Figure 30 shows a group of analytes with structures containing a carbonyl group and a secondary amine group or tertiary amine group.



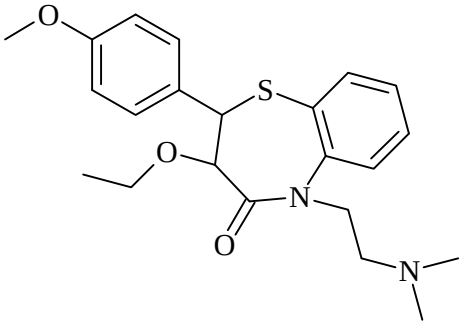
30-1 : ketamine



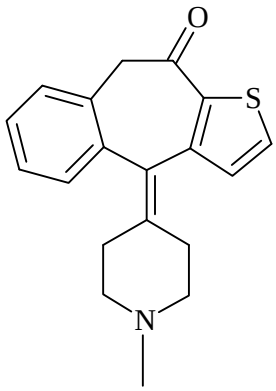
30-2 : bupropion



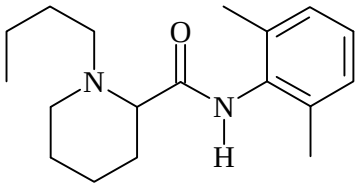
30-3 : tolperisone



30-4 : diltiazem



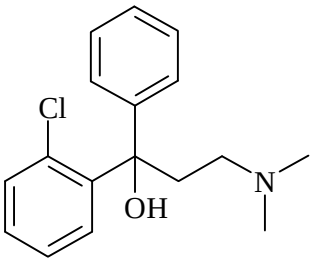
30-5 : ketotifen



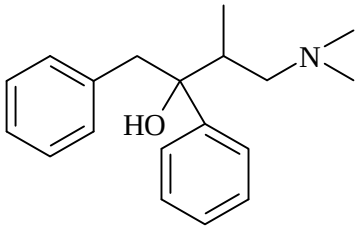
30-6 : bupivacaine

Figure 30. Structures of analytes containing a carbonyl group and secondary or tertiary amino groups.

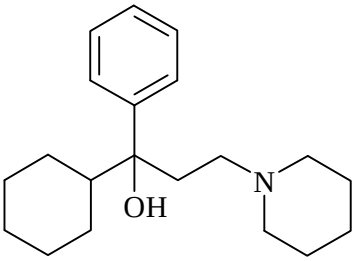
Figure 31 shows a group of analytes with structures containing an alcohol group on a tertiary carbon and a tertiary amino group.



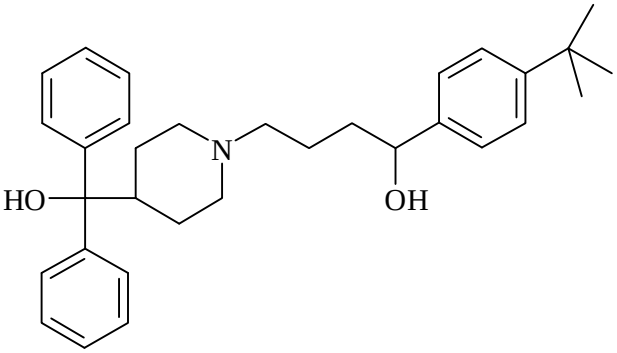
31-1 : chlophedianol



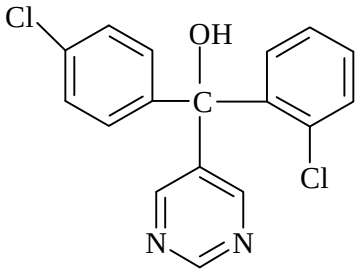
31-2 : oxyphene



31-3 : trihexyphenidyl



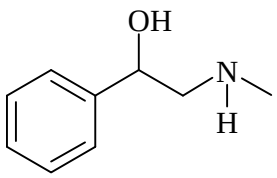
31-4 : terfenadine



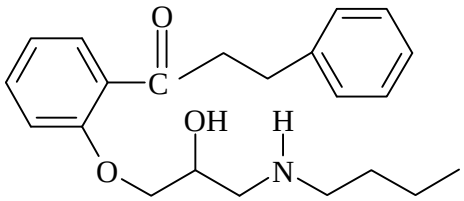
31-5 : fenarimol

Figure 31. Structures of analytes containing an alcohol group on a tertiary carbon and a tertiary amino group.

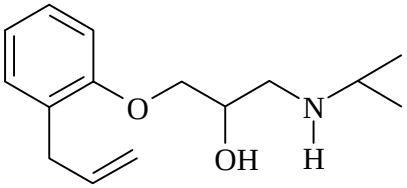
Finally, the structures of analytes containing an alcohol group on a secondary carbon and a secondary amino group are shown in Figure 32.



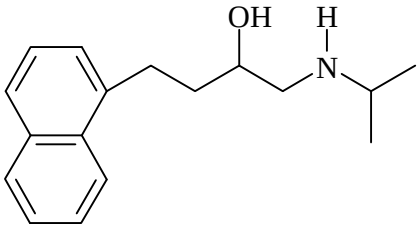
32-1 : halostachine



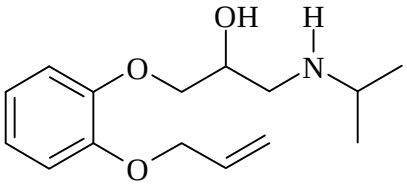
32-2 : propafenone



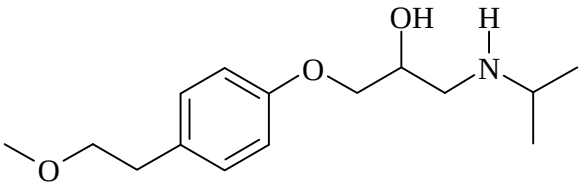
32-3 : alprenolol



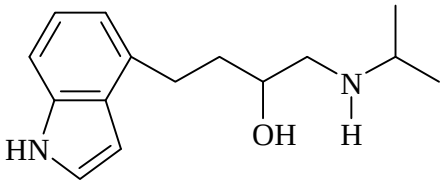
32-4 : propranolol



32-5 : oxprenolol



32-6 : metoprolol



32-7 : pindolol

Figure 32. Structures of analytes containing an alcohol group on a secondary carbon and a secondary amino group.

The first number assigned to the structures corresponds to the number of the figure they are in. The chromatograms for the separation of the drugs are shown in figures 33 to 39.

The first group of amines analyzed contained primary and secondary amine groups (Figure 28). Their chromatograms are shown in Figures 33 - 36. In this figure, 1,2,3,4-tetrahydro-naphthylamine has been assigned as number one for having the primary amine group attached to the tetrahydronaphthyl group, which is the simplest primary amine group. 1-naphthyl-1-ethylamine has the primary amine group at the second carbon atom of a chain group increasing its hindrance. Number 3 is 4-chloramphetamine, which has one more carbon in the alkyl chain than the previous one, and the primary amine group is less exposed than in the previous analyte. Chloroquine, number 4, has a secondary amine that is less exposed than tetrahydrozoline, number 5. The last compound in this series is chlorpheniramine, number 6, which has a tertiary amine group. Some of them, such as 4-chloramphetamine and 1-naphthyl-1-ethylamine, showed small peak tails. The tail is small because the primary amino groups were hindered.

The next group of drugs contain ether groups and secondary or tertiary amino groups (Figure 29). Methoxyphenamine is the simplest compound containing a secondary amine group therefore it has been assigned number 1. Fluoxetine has the same structure as the previous one, but with the addition of a phenyl ring, number 2. Verapamil has a tertiary amine group with a methyl group in it, number 3. Piperoxan has a tertiary amine group that is part of a ring, number 4. Imazalil and miconazole have both nitrogen atoms

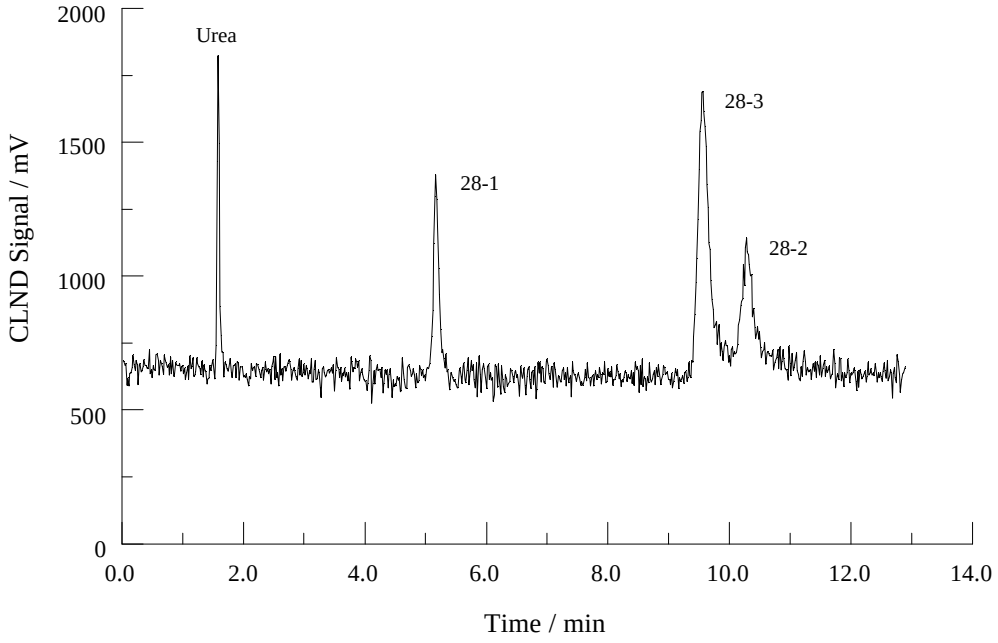


Figure 33. Chromatogram of analytes containing primary amino groups. Peak 28-1:1,2,3,4-tetrahydro-naphthylamine, peak 28-3:4-chloramphetamine, peak 28-2:1-naphthyl-1-ethylamine. Mobile phase:500 mM TMSOH, 15.4 % methanol : 84.6 % water. T=50.5 C.

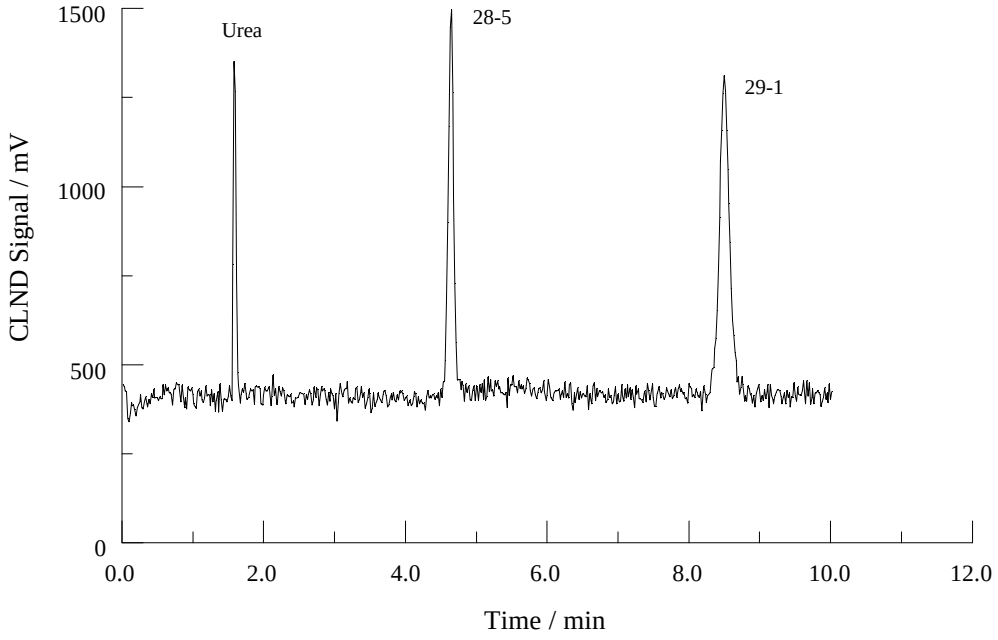


Figure 34. Chromatogram of common drugs containing secondary amino groups. Peak 28-5:tetrahydrozoline, peak 29-1:methoxyphenamine. Mobile phase:500 mM TMSOH, 15.4 % methanol : 84.6 % water. T=50.5 C.

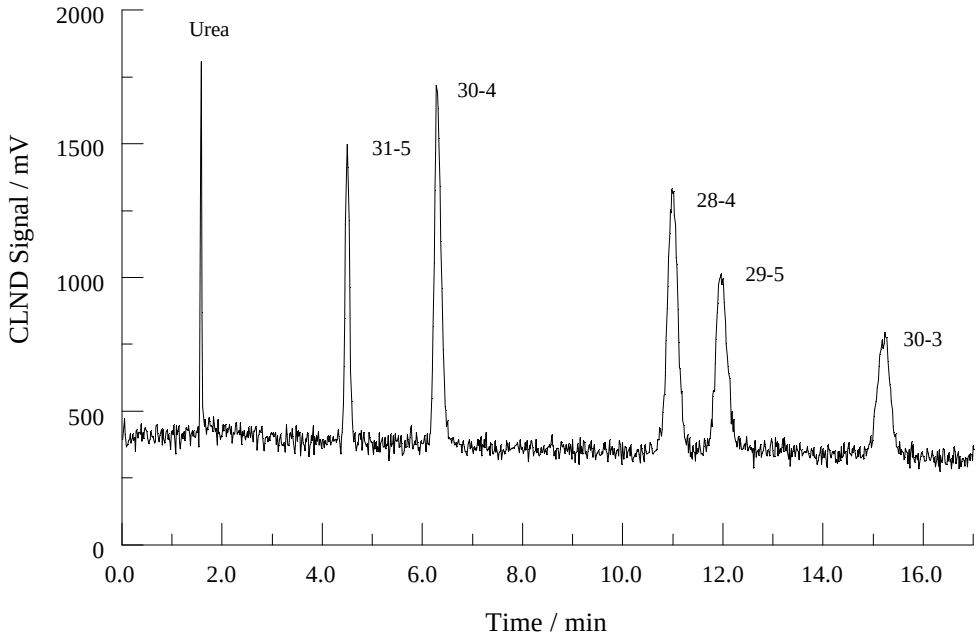


Figure 35. Chromatogram of common drugs containing tertiary amino groups. Peak 31-5:fenarimol, peak 30-4: diltiazem, peak 28-4:chloroquine, peak 29-5:imazalil, peak 30-3:tolperisone. Mobile phase:500 mM TMSOH, 36.5 % methanol : 63.5 % water. T=50.5 C.

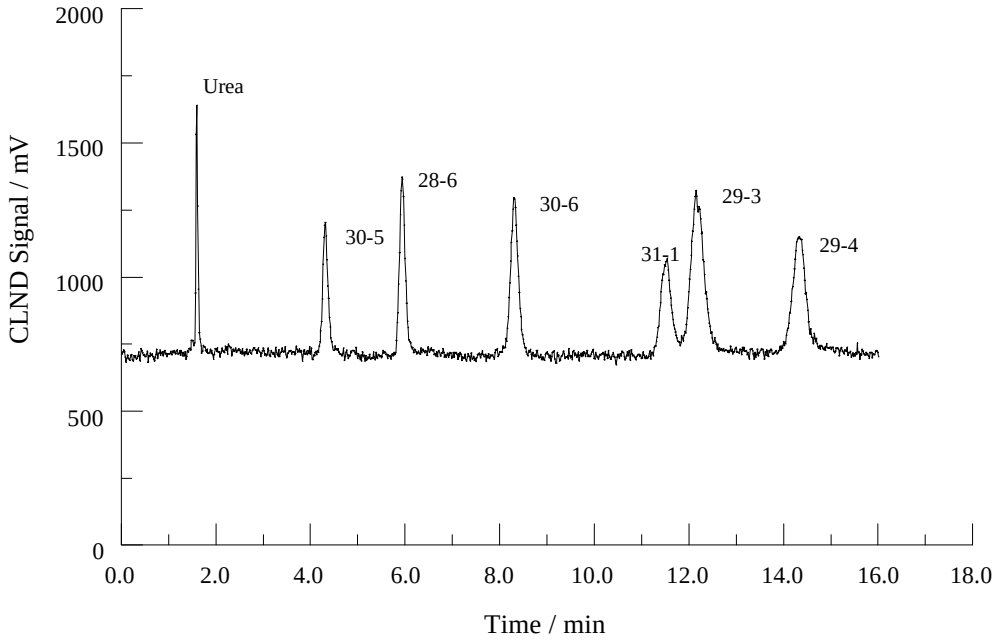


Figure 36. Chromatogram of pharmaceuticals containing tertiary amino groups. Peak 30-5:ketotifen, peak 28-6: chlorpheniramine, peak 30-6:bupivacaine, peak 31-1:chlophedianol, peak 29-3:verapamil, peak 29-4:piperoxan. Mobile phase:500 mM TMSOH, 36.5 % methanol : 63.5 % water. T=50.5 C.

in aromatic rings, imazalil is number 5, and miconazole has a phenyl group extra than imazalil, number 6. Their chromatograms are shown in Figures 34 – 37 and 39. All analytes showed symmetric peaks indicating that bridge formation was not possible. Methoxyphenamine has the lowest retention, fluoxetine has the strongest retention.

The analytes in the next group contained a carbonyl group and a secondary or tertiary amino group (Figure 30). Ketamine has a methyl group attached to the amine group, therefore it has been assigned number 1. Bupropion has a tert-butyl group attached to the amino group, number 2. Tolperisone has a tertiary amino group, number 3. All of them had symmetric peaks as can be seen in Figures 35, 36, 38 and 39. Even though some of the analytes contained a secondary amino group that can interact with the Lewis acid site present in the stationary phase, the additional carbonyl group did not lead to bridging interactions and all peaks were symmetric.

When the analytes shown in Figure 31 were analyzed, they all had symmetric peaks (Figures 35 – 37), even though all had an OH group and a tertiary amino group. Chlophedianol has a tertiary amino group with two methyl groups on the amino group and an alcohol group, number 1. Oxyphene has an extra methyl group and a CH_2 group in between the alcohol group and the phenyl group, number 2. Number 3, trihexyphenidyl, has a tertiary amine group that is part of a ring. Terfenadine, number 4, has one alcohol group on a tertiary carbon and another one on a secondary carbon. Finally, number 5 is fenarimol, with an alcohol group and two nitrogen atoms in an aromatic ring.

The OH^- group containing samples were also analyzed by capillary electrophoresis using the mobile phase with which the chromatograms were obtained as

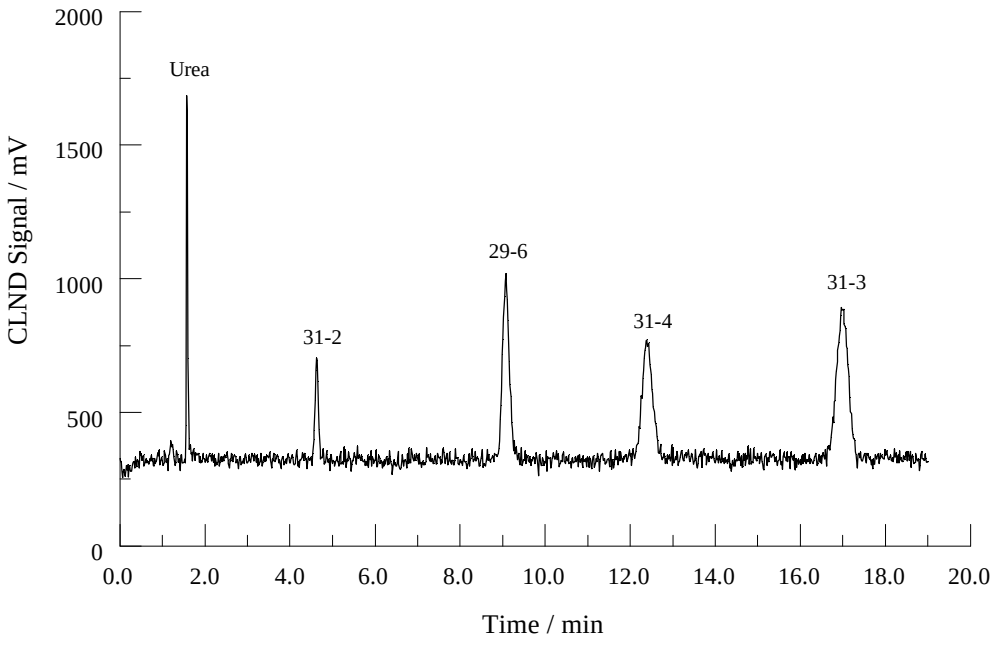


Figure 37. Chromatogram of common drugs containing tertiary amino groups. Peak 31-2:oxyphene, peak 29-6: miconazole, peak 31-4:terfenadine, peak 31-3:trihexyphenidyl. Mobile phase:500 mM TMSOH, 57.3 % methanol : 42.7 % water. T=55.0 C.

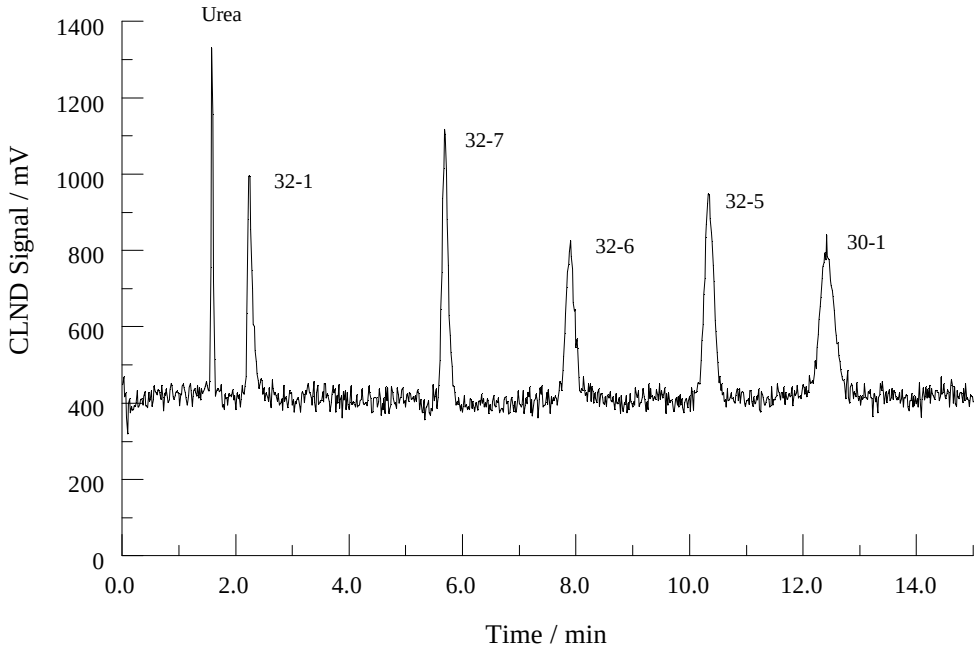


Figure 38. Chromatogram of common drugs containing secondary amino groups. Peak 32-1:halostachine, peak 32-7:pindolol, peak 32-6:metoprolol, peak 32-5:oxprenolol, peak 30-1:ketamine. Mobile phase:500 mM TMSOH, 15.4 % methanol : 84.6 % water. T=50.5 C.

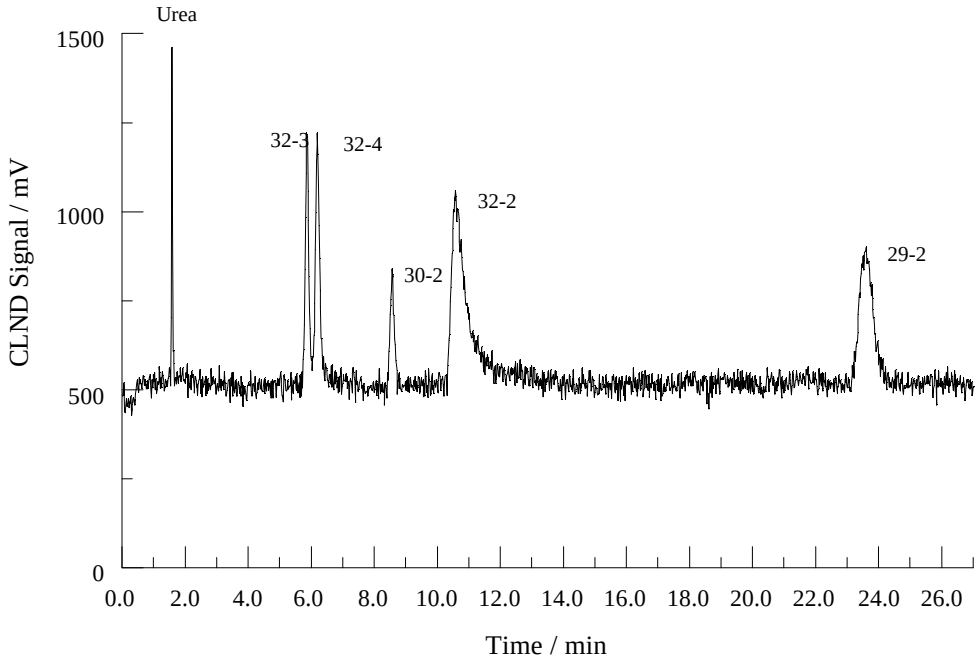


Figure 39. Chromatogram of common drugs containing secondary amino groups. Peak 32-3:alprenolol, peak 32-4: propranolol, peak 30-2:bupropion, peak 32-2:propafenone, peak 29-2:fluoxetine. Mobile phase:500 mM TMSOH, 36.5 % methanol : 63.5 % water. T=50.5 C.

the background electrolyte. Most of the analytes were negatively charged. The mobilities found were -1.21×10^{-5} cm^2/Vs for chlophedianol, -4.26×10^{-5} cm^2/Vs for fenarimol and -3.02×10^{-5} cm^2/Vs for oxyphene. For trihexyphenidyl and terfenadine, the mobility values found were so small that they could not be determined accurately. Therefore, it was assumed that both compounds were neutral when analyzed by CE and HPLC. Since the analytes had a tertiary amino group, they could not interact strongly with the Lewis acid sites in the stationary phase, despite the presence of the possibly dissociated OH group.

The last group of analytes containing both an OH group on a secondary carbon and a secondary amino group (Figure 32) gave different results. All the compounds in this group have both an alcohol group and an amino group separated by a CH_2 group. Halostachine has a methyl group attached to the amino group, number 1. Propafenone has an n-butyl group attached to the amino group, number 2. The rest of the structures have an isopropyl group attached to the amino group. Number 3, alprenolol additionally has an allyl phenyl ether group, number 4, propranolol a naphthyl group. Oxprenolol is number 5 and has two ether groups close to each other, number 6 is metoprolol with two ether groups far from each other. Finally, pindolol, with another amino group, is number 7. Though there was a possibility of forming a bridge interaction with their Lewis base groups and the Lewis acid sites present in the stationary phase, most of them had symmetric peaks (Figures 38 and 39), except propafenone that showed a long tail and halostachine that showed a small tail.

All these drugs were analyzed by capillary electrophoresis to find out whether the OH groups in their structure were ionized or not under the HPLC separation conditions.

The mobile phase used to obtain the chromatograms was used as the background electrolyte in capillary electrophoresis. The results showed that all the analytes had an anionic effective mobility, i.e., a negative charge. However, only propafenone and halostachine showed a tail. Propafenone had a mobility of -2.91×10^{-5} cm^2/Vs and halostachine had a mobility of -1.85×10^{-5} cm^2/Vs. As for the other amines, the mobilities found were -1.00×10^{-5} cm^2/Vs for propranolol, -2.19×10^{-5} cm^2/Vs for alprenolol, -1.19×10^{-5} cm^2/Vs for metoprolol, -1.25×10^{-5} cm^2/Vs for oxprenolol and -1.00×10^{-5} cm^2/Vs for pindolol.

Propafenone and halostachine possibly showed a tail because the alkyl group present in the amino group is an n-butyl and a methyl group, respectively that allowed the amino groups to interact with the stationary phase and form the bridge interaction with both OH group and amino group. The rest of the analytes have isopropyl groups on the NH group that hinder the bridge formation with between the Lewis bases present in the structure and the Lewis acid sites present in the stationary phase.

3.8 Analysis of Amino Acids and Amino Phenols

In the 500 mM TMSOH eluents, both the carboxylic and the amino groups of amino acids were deprotonated. Because of this, their solubility in the mobile phase was high and their retention was weak.

For this analysis, it was thought to use amino acids that would have higher hydrophobicity and therefore higher retention on the stationary phase. The amino acids tested were tryptophan, phenylalanine, proline, leucine, isoleucine and tyrosine. Figure 40 shows the chromatograms of the amino acids, the mobile phase used was 500 mM TMSOH, 5.1 % methanol and 94.9 % water.

As can be seen from their chromatograms, all amino acids tested had a very small interaction with the stationary phase, phenylalanine eluted with the urea while the other samples eluted before urea.

The amino phenols analyzed were salbutamol, synephrine, methanephrine, metaproterenol and labetalol, their structures are shown in Figure 41. Figure 42 shows chromatograms of the analytes when analyzed with a 500 mM TMSOH, 5.1 % methanol, 94.9 % water mobile phase. As can be seen, salbutamol had the highest retention of all amino phenols tested, which eluted with urea, all other analytes had a retention smaller than urea, because the phenoloc OH groups were fully deprotonated in the 500 mM TMSOH solutions.

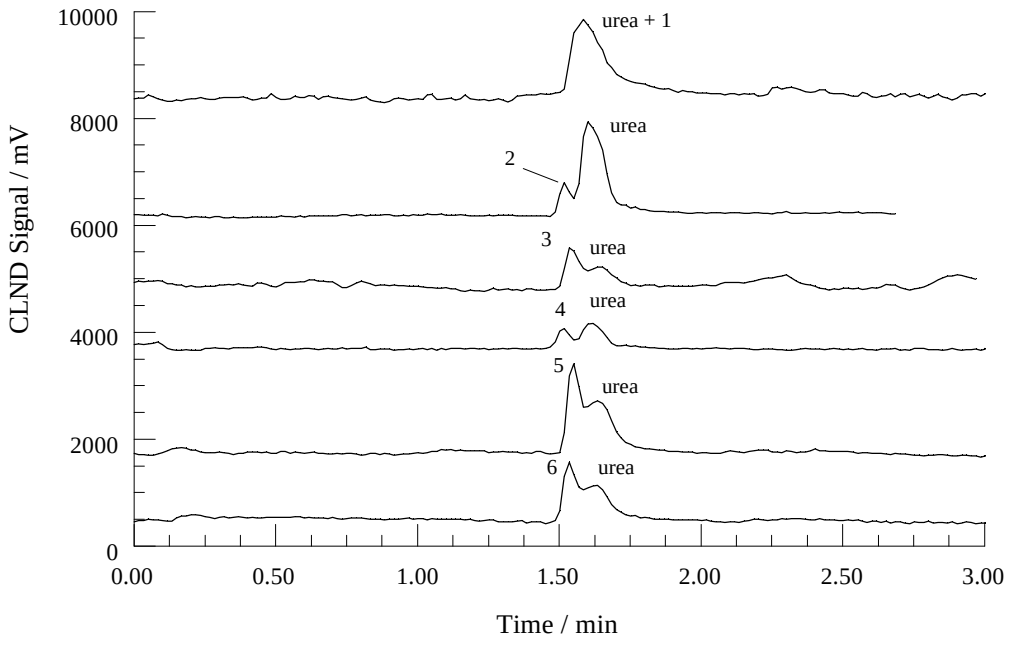
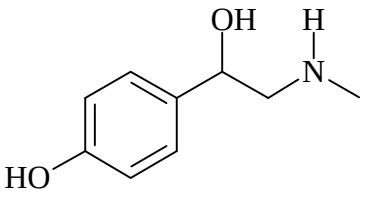
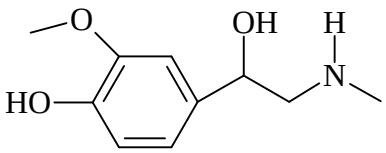


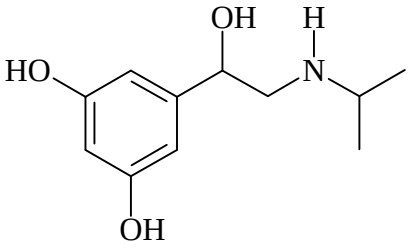
Figure 40. Chromatograms of amino acids. Peak 1:phenylalanine, Peak 2:tryptophan, Peak 3:tyrosine, Peak 4:proline, Peak 5:leucine, Peak 6:isoleucine. Mobile phase:500 mM TMSOH, 5.1 % methanol : 94.9 % water. T=50.5 C.



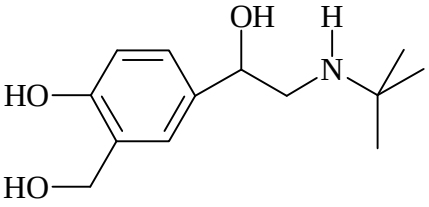
synephrine



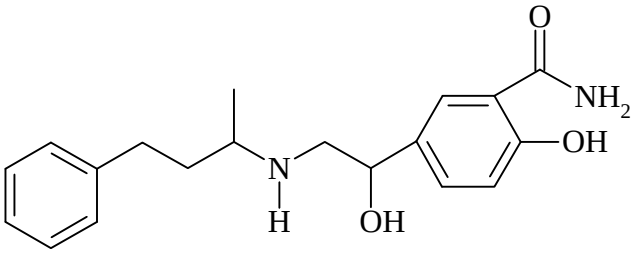
methanephrine



metaproterenol



salbutamol



labetalol

Figure 41. Structures of amino phenols.

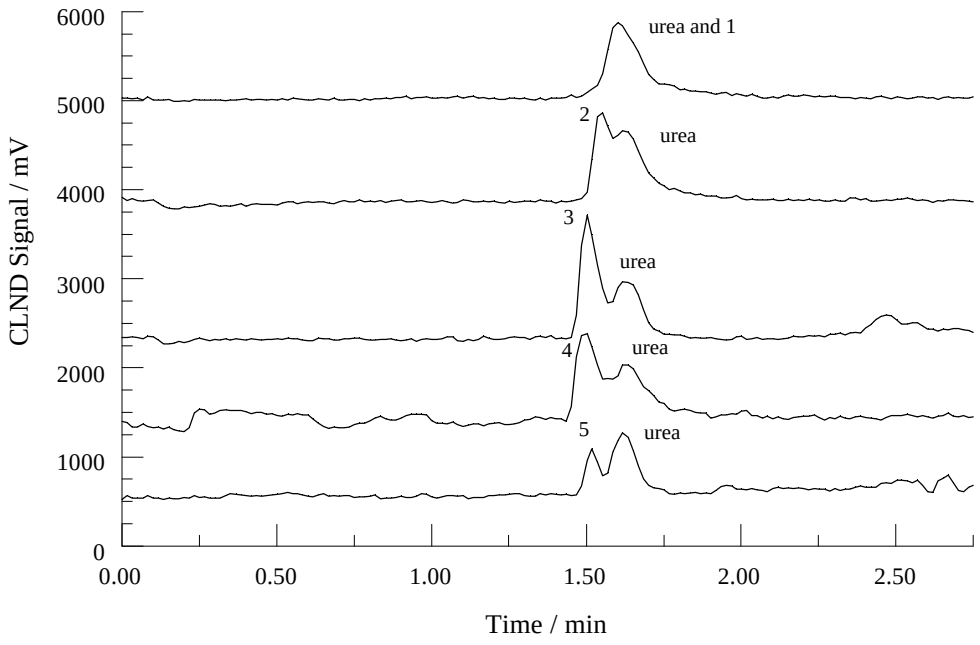


Figure 42. Chromatograms of amino phenols. Peak 1:salbutamol, Peak 2:synephrine, Peak3::metanephrine, Peak 4:metaproterenol, Peak 5:labetalol. Mobile phase:500 mM TMSOH, 35.6 % methanol : 63.5 % water. T=50.5 C.

3.9 Universal Calibration Curve of Detection

The CLND has the unique ability to utilize a single calibration curve for quantification of analytes. To corroborate this in the system used, a calibration curve was obtained for a typical amine from each of the amine groups studied, both in the FIA (flow injection analysis) mode and in the HPLC mode (with the PBD-zirconia column).

Figure 43 shows the calibration curve of the CLND in the FIS-mode for all the analytes tested: tetrapropylammonium cation, tripropylamine, dipropylamine, 2-butylamine, butylamine and 2-amino-3-phenyl-1-propanol.

A regression line was obtained for every calibration curve of the separated amine samples tested and it was found that all of them had a linear response (r^2) of 0.999. Linearity was observed over two orders of magnitude from 0.1 nmoles to 10 nmoles. The calibration curves have similar slope values (3.92×10^9 with a standard deviation of 0.07×10^9), which indicates the CLND system provides a universal response that allows for a single calibration curve of quantification.

However, when the zirconia based-column was coupled to the CLND, a different calibration curve was obtained (Figure 44). Linearity was observed from 0.5 nmoles to 100 nmoles for tetrapropylammonium cation and sec-butylamine, from 5 nmoles to 100 nmoles for tripropylamine, dipropylamine and butylamine and from 34.5 nmoles to 100 nmoles for 2-amino-3-phenyl-1-propanol. All samples were run in triplicates.

Tetrapropylammonium cation, tripropylamine, dipropylamine and sec-butylamine have shown similar peak areas for the same number of moles of analytes injected. The r^2 value found for these analytes was 0.999 with a slope of 3.55×10^9, which seems to

suggest that the analytes have similar adsorption/desorption characteristics on the PBD-zirconia column, even for the sec-butylamine that shows a moderate peak tail (Chapter 3.5). However, butylamine, that shows a pronounced peak tail, showed a slope lower than the previous set of amines ($3.19 \text{ X } 10^9$, $r^2 = 0.999$), which would seem to suggest that the adsorption/desorption characteristics on the column are different than that of the amines just mentioned and some of the analyte is retained on the surface of the stationary phase. In the case of 2-amino-3-phenyl-1-propanol, the slope of the calibration curve is $2.77 \text{ x } 10^9$, $r^2 = 0.99$, a value much lower than found for the other amines, suggesting that a bigger amount of the analyte injected is retained on the stationary phase.

All experiments were done using a mobile phase that gave k' values in the $0.45<k'<2.6$ range, i.e. a mobile phase with 500 mM TMSOH, 5.1 % methanol and 94.9 % water, except when using tripropylamine where the mobile phase used was 46.9 % methanol and 53.1 % water and gave a k' of 1.45. The injection volume used was 1 ìL, the temperature 51 C.

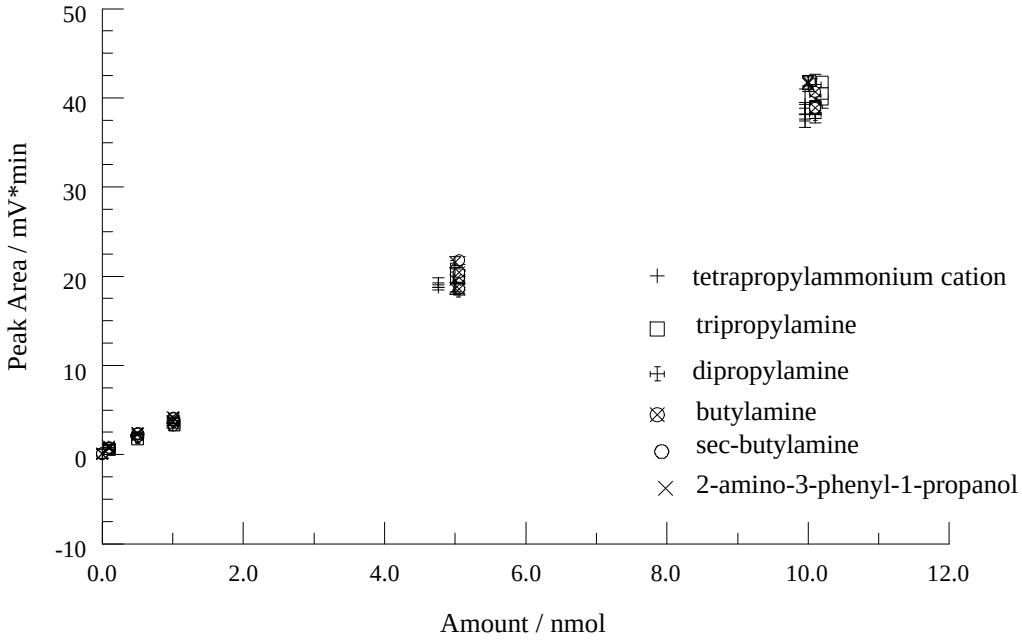


Figure 43. Universal calibration curve of CLND in the FIA-mode.

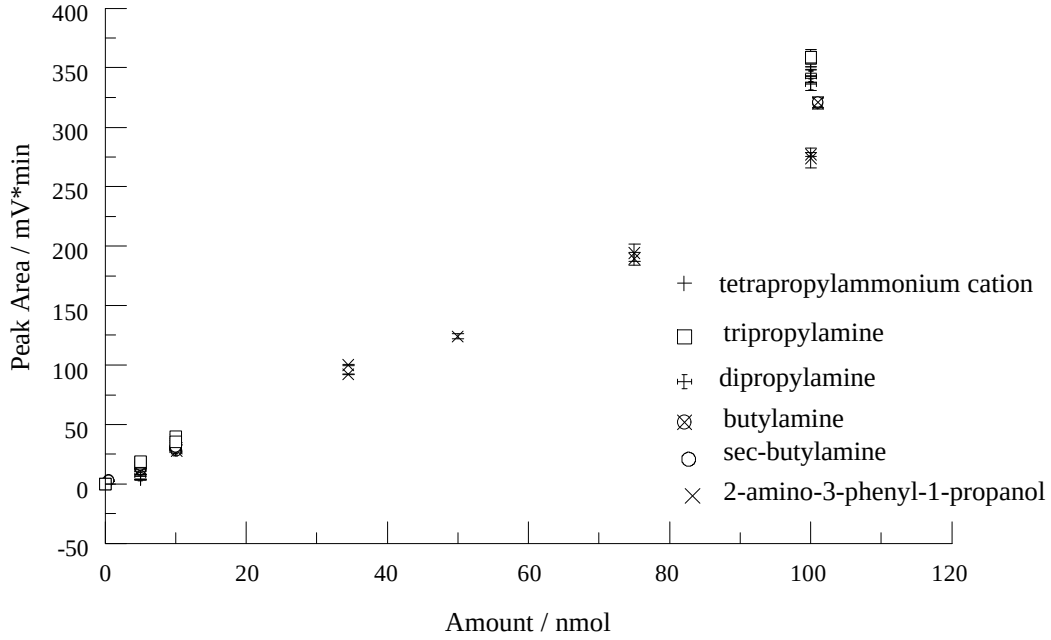


Figure 44. Calibration curve of CLND in the HPLC-mode with the PBD-zirconia column.

CHAPTER IV

CONCLUSIONS

Trimethylsulfonium hydroxide (TMSOH) has been introduced as a new strong electrolyte base with volatile combustion products to replace the alkali metal hydroxides that when used in the CLND for long periods of time, foul the detector.

The present research targeted amines that are mostly analyzed by ion pair reversed-phase HPLC. However, ion-pair reversed-phase HPLC is a complicated system and it was avoided in this investigation. To do so, the amines had to be analyzed in their deprotonated form to increase their interaction with the reversed-phase stationary phase. This could only be accomplished using a mobile phase that had pH much higher than the pKa values of the amines. However, the commonly used silica-based columns are not stable at pH higher than 8, therefore an alternative stationary phase had to be found.

The alternative to silica-based columns was the zirconia-based columns, which are stable up to pH 14. This column was used through out the entire investigation.

It was found that 500 mM TMSOH added to the eluent was suitable for the analysis of the amines tested. At that concentration, interaction of the amines with bare patches of zirconia was low and the plate number was high as shown in Table 1 (CHAPTER IV).

UV transparent quaternary amines were analyzed. They showed an overall small retention on the stationary phase since they have a positive charge and a high solubility. In spite of that, the log k’ vs. carbon number plots indicated that the main interaction

between the quaternary amines and the stationary phase was hydrophobic. UV absorbing quaternary amines were also analyzed. However, the linearity expected in the log k' vs. carbon number (Cn) plot was not observed. The conclusion drawn from that was that the analytes could not fully interact with the hydrophobic stationary phase.

Symmetric and non-symmetric tertiary UV transparent amines were also analyzed, they showed higher retention than the quaternary amines and it was concluded that the main interaction between them and the stationary phase was hydrophobic in nature.

UV absorbing secondary amines were analyzed. They showed that for the shorter alkyl chains, the amino group was able to interact with the Lewis acid sites of the stationary phase. This interaction caused a peak tail where the alkyl chain present on the amino group was such as a longer butyl group, the analyte could not readily interact with the Lewis acid sites of the stationary phase and caused strong peak tailing.

The last type of amino group to analyze was the primary amino group. The results showed long peak tails for all primary amines tested. This was because the primary amino group is a hard Lewis base that can interact with the exposed hard Lewis acid sites on the zirconia-based stationary phase. When the amino group was moved from the end on the alkyl chain to the middle of the alkyl chain, the peak tail decreased considerably. This was because the alkyl backbone hindered the interaction of the amino group with the Lewis acid sites present on the zirconia stationary phase. The peak tail decreased the most when the amino group was moved to the 3rd or 4th carbon of the alkyl chain.

Compounds containing both an alcohol group and an amino group in their structure were then analyzed. The results obtained from these studies showed that both

groups interact with the exposed Lewis acid sites on the stationary phase to form a bridge interaction. This bridge interaction was more pronounced for compounds that contained a primary amino group along with the alcohol group. Compounds with secondary amines and an alcohol group showed a smaller tail than the previous combination of primary amino groups and an alcohol group. This was because in the secondary amino groups, there was an extra alkyl chain on the nitrogen atom that could hinder close approach of the Lewis acid site. Capillary electrophoretic experiments showed that all of these analytes had a negative charge under conditions at which they were run in HPLC, therefore the alcohol group were deprotonated.

In the case of a compound having both an alcohol group and a tertiary amino group, peak tail was not observed. This was because a tertiary amine is not a hard Lewis base and it can not interact with the Lewis acid site from the stationary phase. From this it can be concluded that peak tailing was aggravated when two hard Lewis bases were present in a compound that could interact with the Lewis acid sites of the stationary phase.

After the model amino compounds were tested, some common drugs were studied. The results showed similar conclusions. When tertiary amine groups were present in the compounds, peaks were symmetric. The samples that contained both a tertiary amino group and an alcohol group also showed symmetric peaks. When the compounds had a secondary amino group and an alcohol group, peak shape varied. Propafenone showed a long tail because the alkyl group present on the amino group was an n-butyl group, which allowed some bridge interaction between the analyte and the stationary phase. Halostachine showed a tail as well, because the amino group had a

methyl group attached to it, allowing bridge interaction. The rest of the compounds have an isopropyl group attached to the amino group that inhibits bridge formation and the peak shape observed for them was symmetric. Some of the compounds that contained only amino groups showed symmetric peaks where the amino group was hindered and some of them showed a peak tail where the amino group was somewhat exposed.

Some amino acids and amino phenols were also tested. However, no success was obtained as these analytes had a very high solubility in the mobile phase and were extremely weakly retained on the stationary phase.

It has been proven that the CLND detector has a universal response having a universal calibration curve, as demonstrated by the detection of six different analytes in the FIA-mode. However, in the HPLC-mode, the universality is observed for analytes that have similar adsorption/desorption rates on the stationary phase such as, quaternary, tertiary, secondary and hindered-primary amines that were represented in this investigation by tetrapropylammonium cation, tripropylamine, dipropylamine and sec-butylamine, respectively. However, in the case of exposed primary amines, the adsorption/desorption rate from the stationary phase is lower, giving a lower response if compared to the previous analytes. This is observed because part of the analyte is retained on the stationary phase. A sample test combining both alcohol and amino groups, both hard Lewis acid sites, showed the lowest response of all (2-amino-3-pehyl-1-propanol) indicating that there is a bigger amount of analyte retained on the stationary phase. Therefore, in the system the universality of the response is mantained as long as the adsorption/desorption rate of different analytes is similar.

REFERENCES

[1] L.R. Snyder, J.J. Kirkland, J.L. Glajch. Practical HPLC Method Development, second edition, New York. 1997.

[2] A. Wejrli, J.C. Hildebrand, H.P. Keller, R. Stampfli, R.W. Frei. J. Chromatogr. 149 (1978) 199.

[3] J.L. Glajch, J.J. Kirkland, J. Kohler. J. Chromatogr. 384 (1987) 81.

[4] N. Tanaka, T. Tanigawa, K. Kimata, K. Hosoya, T. Araki. J. Chromatogr. 549 (1991) 29.

[5] H.A. Claessens, M.A. van Straten, J.J. Kirkland, J. Chromatogr. A 728 (1996) 259.

[6] C. Laurent, H.A.H. Billiet, L. de Galan. Chromatographia 17 (1983) 253.

[7] J. Kriz, E. Adamcova, J.H. Knox, J. Hora. J. Chromatogr. A 663 (1994) 151.

[8] M.P. Rigney, E.F. Funkenbusch, P.W. Carr. J. Chromatogr. 499 (1990) 291.

[9] C.J. Dunlap, C.V. McNeff, D. Stoll, P.W. Carr. Anal. Chem. 73 (2001) 598A.

[10] M. Kawahara, H. Nakamura, T. Nakajima. J. Chromatogr. 515 (1990) 149.

[11] U. Trudinger, G. Muller, K.K. Unger. J. Chromatogr. 535 (1990) 111.

[12] J.H. Knox, B. Kaur, G.R. Millward. J. Chromatogr. 352 (1986) 3.

[13] M.P. Rigney, T.P. Weber, P.W. Carr. J. Chromatogr. 484 (1989) 273.

[14] T.P. Weber, P.W. Carr. Anal. Chem. 62 (1990) 2620.

[15] T.P. Weber, P.W. Carr, E.F. Funkenbusch. J. Chromatogr. 519 (1990) 31.

[16] J. Nawrocki, M.P. Rigney, A. McCormick, P.W. Carr. J. Chromatogr. A 657 (1993) 229.

[17] J. Li, P.W. Carr. Anal. Chem. 68 (1996) 2857.

[18] J. Li, P.W. Carr. Anal. Chim. Acta 334 (1996) 239.

[19] J. Li, D.H. Reeder, A.V. McCormick, P.W. Carr. J. Chromatogr. A 791 (1997) 45.

[20] J. Li, P.W. Carr. Anal. Chem. 69 (1997) 2193.

[21] J. Li, Y. Hu, P.W. Carr. Anal. Chem. 69 (1997) 3884.

[22] P.T. Jackson, T.-Y. Kim, P.W. Carr. Anal. Chem. 69 (1997) 5011.

[23] Y. Hu, P.W. Carr. Anal. Chem. 70 (1998) 1934.

[24] L.C. Tan, P.W. Carr. J. Chromatogr. A 799 (1998) 1.

[25] M. Reta, P.W. Carr. J. Chromatogr. A 855 (1999) 121.

[26] Y. Mao, P.W. Carr. Anal. Chem. 73 (2001) 1821.

[27] P. T. Jackson, P.W. Carr. J. Chromatogr. A 958 (2002) 121.

[28] Y. Xiang, B. Yan, B. Yue, C.V. McNeff, P.W. Carr, M.L. Lee. J. Chromatogr. A 983 (2003) 83.

[29] X. Yang, J. Dai, P.W. Carr. Anal. Chem. 75 (2003) 3153.

[30] J.A. Blackwell, P.W. Carr. J. Chromatogr. 549 (1991) 43.

[31] J.A. Blackwell, P.W. Carr. J. Chromatogr. 549 (1991) 59.

[32] W.A. Schafer, P.W. Carr, E.F. Funkenbusch, K.A. Parson. J. Chromatogr. 587 (1991) 137.

[33] J.A. Blackwell, P.W. Carr. Anal. Chem. 64 (1992) 853.

[34] J.A. Blackwell, P.W. Carr. Anal. Chem. 64 (1992) 863.

[35] W.J. Cheong, P.W. Carr. J. Liq. Chrom. 10 (1987) 561.

[36] C. McNeff, Q. Zhao, P.W. Carr. J. Chromatogr. A 684 (1994) 201.

[37] L.C. Tan, P.W. Carr, J.M.J. Frecht, V. Smigol. Anal. Chem. 66 (1994) 450.

[38] B.C. Trammell, M.A. Hillmyer, P.W. Carr. Anal. Chem. 73 (2001) 3323.

[39] Y. Hu, X. Yang, P.W. Carr. J. Chromatogr. A 968 (2002) 17.

[40] Y. Hu, P.W. Carr. Chromatographia 56 (2002) 439.

[41] X. Yang, J. Dai, P.W. Carr. J. Chromatogr. 996 (2003) 13.

[42] J. Dai, X. Yang, P.W. Carr. J. Chromatogr. 1005 (2003) 63.

[43] R.J.Kok, J. Visser, F. Moolenaar, D. de Zeeuw, D.K.F. Meijer. J. Chromatogr. B 693 (1997) 181.

[44] A. Taga, A. Nishino, S. Honda. J. Chromatogr. A 822 (1998) 271.

[45] D.F. Chollet, L. Goumaz, C. Juliano, G. Anderegg. J. Chromatogr. 746 (2000) 311.

[46] K.B. Male, J.H.T. Luong. J. Chromatogr. A 926 (2001) 309.

[47] X. Liu, L.H. Takahashi, W.L. Fitch, G. Rozing, C. Bayle, F. Couderc. J. Chromatogr. A 924 (2001) 323.

[48] R. Bartzatt. J. Pharm. and Toxic. Meth. 45 (2001) 247.

[49] H. Wang, J. Li, X. Liu, T.-X. Yang, H.-S. Zhang. Anal. Biochem. 281 (2000) 15.

[50] H. Wang, J. Li, X. Liu, T.-X. Yang, H.-S. Zhang. Anal. Chimica Acta 423 (2000) 77.

[51] R.M. Latorre, S. Hernandez-Cassou, J. Saurina. J. Chromatogr. A 934 (2001) 105.

[52] R. Driouich, T. Takayanagi, M. Oshima, S. Motomizu. J. Chromatogr. A 934 (2001) 95.

[53] R. Driouich, T. Takayanagi, M. Oshima, S. Motomizu. J. Pharm. and Biomed. Anal. 30 (2003) 1523.

[54] J. Morley, L. Elrod Jr., C. Linton, D. Shaffer, S. Krogh. J. Chromatogr. A 766 (1997) 77.

[55] N. Wad, G. Kramer. J. Chromatogr. B 705 (1998) 154.

[56] Z. Chen, J. Wu, G.B. Baker, M. Parent, N.J. Dovichi. J. Chromatogr. A 914 (2001) 293.

[57] S. Erturk, E.S. Aktas, S. Atmaca. J. Chromatogr. B 760 (2001) 207.

[58] Y. Zhang, F.A. Gomez. Electrophoresis 21 (2000) 3305.

[59] A. M. Garcia-Campana, W.R.G. Baeyens. Chemiluminescence in Analytical Chemistry, New York. 2001.

[60] J.W. Birks. Chemiluminescence and Photochemical Reaction Detection in Chromatography. Wiley-VCH, New York 1989.

[61] M.L. Grayeski. Anal. Chem. 59 (1987) 1243A.

[62] T.G. Burdo, W.R. Seitz. Anal. Chem. 47 (1975) 1643.

[63] W.R. Seitz. J. Phys. Chem. 79 (1975) 101.

[64] A. MacDonald, T.A. Neiman. Am. Chem. Soc. 57 (1985) 936.

[65] S.N. Brune, D.R. Bobbitt. Talanta 38 (1991) 419.

[66] S.N. Brune, D.R. Bobbitt. Anal. Chem. 64 (1992) 166.

[67] W.A. Jackson, D.R. Bobbitt. Anal. Chimica Acta 285 (1994) 309.

[68] X. Wang, D.R. Bobbitt. Anal. Chimica Acta 383 (1999) 213.

[69] R.J. VanZee, A.U. Khan. J. Chem. Phys. 65 (1976) 1764.

[70] R.J. VanZee, A.U. Khan. J. Phys. Chem. 80 (1976) 2240.

[71] D.G. Harris, M.S. Chou, T.A. Cool. J. Chem. Phys. 82 (1985) 3502.

[72] R.L. Benner, D.H. Stedman. Am. Chem. Soc. 61 (1989) 1268.

[73] H.-C. K. Chang, L.T. Taylor. Anal. Chem. 63 (1991) 486.

[74] T.B. Ryerson, A.J. Dunham, R.M. Barkley, R.E. Sievers. Anal. Chem. 66 (1994) 2841.

[75] X. Yan. J. Chromatogr. A 976 (2002) 3.

[76] A. Sokolowski. Ph. D. Dissertation. Texas A&M University. 2000.

[77] E.M. Fujinari, L.O. Courthaudon. J. Chromatogr. 592 (1992) 209.

[78] E.M. Fujinari. Develop. Food Sci. 32 (1993) 31.

[79] E.M. Fujinari, J.D. Manes. Develop. Food Sci. 37A (1995) 929.

[80] E.M. Fujinari, E. Ribble, M.V. Piseichio. Develop. Food Sci. 32 (1993) 75.

[81] R. Bizanek, J. Damon, M. Fujinari, E.M. Fujinari. Pep. Res. 9 (1996) 40.

[82] E.M. Fujinari. Develop. Food Sci. 32 (1993) 55.

[83] E.M. Fujinari. Develop. Food Sci. 33 (1993) 1033.

[84] E.M. Fujinari. Develop. Food Sci. 34 (1994) 367.

[85] E.M. Fujinari. ACS Sym. Series 660 (1997) 98.

[86] E.M. Fujinari. Develop. Food Sci. 37A (1995) 379.

[87] H. Shi, L.T. Taylor, E.M. Fujinari. J. High Res. Chrom. 19 (1996) 213.

[88] E.M. Fujinari, J.D. Manes. J. Chromatogr. A 676 (1994) 113.

[89] H. Shi, J.T.B. Strode, L.T. Taylor, E.M. Fujinari. J. Chromatogr. A 734 (1996) 303.

[90] H. Shi, L.T. Taylor, E.M. Fujinari. J. Chromatogr. A 757 (1997) 183.

[91] H. Shi, L.T.B Thompson, L.T. Taylor, E.M. Fujinari. Develop. Food Sci. 39 (1998) 475.

[92] E.M. Fujinari, J.D. Manes. J. Chromatogr. A 763 (1997) 323.

[93] F.J. Kolpak, J.E. Brady, E.M. Fujinari. Develop. Food Sci. 39 (1998) 467.

[94] E.W. Taylor, M.G. Qian, G.D. Dollinger. Anal. Chem. 70 (1998) 3339.

[95] K. Lewis, D. Phelps, A. Sefler. Am. Pharm. Review 3 (2000) 63.

[96] D.A. Yurek, D.L. Branch, M.-S. Kuo. J. Comb. Chem. 4 (2002) 138.

[97] N. Shah, M. Gao, K. Tsutsui, A. Lu, J. Davis, R. Scheuerman, W.L. Fitch, R.L. Wilgus. J. Combin. Chem. 2 (2000) 453.

[98] D. Brannegan, M. Ashraf-Khorassani, L.T. Taylor. J. Chrom. Sci. 39 (2001) 217.

[99] C.A. Lucy, C.R. Harrison. J. Chromatogr. A 920 (2001) 135.

[100] K. Petritis, C. Elfakir, M. Dreux. LC-GC 14 (2001) 389.

[101] M.A. Nussbaum, S.W. Baertschi, P.J. Jansen. J. Pharm. Biomed. Sci. 27 (2002) 983.

[102] K. Petritis, C. Elfakir, M. Dreux. J. Chromatogr. A 961 (2002) 9.

[103] C.R. Harrison, C.A. Lucy. J. Chromatogr. A 956 (2002) 237.

[104] C.G. Swain, L.E. Kaiser. J. Am. Ch. Soc. 80 (1958) 4089.

[105] C.G. Swain, L.E. Kaiser, T.E.C. Knee. J. Am. Ch. Soc. 80 (1958) 4092.

[106] E.D. Hughes, C. Ingold, Y. Pocker. Chem. Ind. (1959) 1282.

[107] E.D. Hughes, D.J. Whittingham. J. Chem. Soc. (1960) 806.

[108] T.W. Milligan, B.C. Minor. J. Org. Chem. 28 (1963) 235.

[109] G. Beurskens, G.A. Jeffrey, R.K. McMullan. J. Chem. Phys. 39 (1963) 3311.

[110] J.B. Hyne, H.S. Golinkin. Can. J. Chem. 41 (1963) 3139.

[111] Y. Pocker, A.J. Parker. J. Org. Chem. 31 (1966) 1526.

[112] C.G. Swain, W.D. Burrows, B.J. Schowen. J. Org. Chem. 33 (1968) 2534.

[113] W.R.T. Cottrell, R.A.N. Morris. Chem. Comm. 212 (1968) 409.

[114] S. Lindenbaum. J. Phys. Chem. 72 (1968) 212.

[115] D.F. Evans, T.L. Broadwater. J. Phys. Chem. 72 (1968) 1037.

[116] A.A. Sosunov, A.P. Kilimov, V.V. Smirnov. Zhur. Obs. Khim. 40 (1970) 2688.

[117] G.A. Olah, A.T. Ku, J.A. Plah. J. Org. Chem. 35 (1970) 3904.

[118] I.P. Samchenko, L.A. Kirpianova. Zhur. Org. Khim. 8 (1972) 2220.

[119] A.A. Sosunov, A.P. Kilimov, V.V. Smirnov. Zhur. Obs. Khim. 45 (1975) 1533.

[120] R.T. Hargreaves, A.M. Katz, W.H. Saunders. J. Am. Chem. Soc. 98 (1976) 2614.

[121] M.N. Islam, K.T. Leffek. J. Chem. Soc. Perkin II. (1977) 968.

[122] K. Yamauchi, T. Tanabe, M. Kinoshita. J. Org. Chem. 44 (1979) 638.

[123] W. Butte, J. Eilers, M. Oldenburg. Anal. Lett. 15 (1982) 841.

[124] S. Kondo, Y. Takeda, K. Tsuda. Commun. (1988) 403.

[125] D.P. Phillion, S.S. Andrew. Tetrahedron Lett. 30 (1991) 3621.

[126] Y.B. Fan, D.J. Donaldson. J. Phys. Chem. 96 (1992) 19.

[127] N. Ohmori, Y. Nakazomo, M. Hata, T. Hoshino, M. Tsuda. J. Phys. Chem. 102 (1998) 927.

[128] H. Matsumoto, T. Matsuda, Y. Miyazaki. Chem. Lett. (2000) 1430.

[129] R.R. Donnelley. Dowex:Ion exchange. The Lakeside Press, Michigan City 1959

[130] B.A. Williams, G. Vigh. Anal. Chem. 68 (1996) 1174.

[131] A. Bartha, G. Vigh. J. Chromatogr. 260 (1983) 337.

[132] A. Bartha, G. Vigh. J. Chromatogr. 265 (1983) 171.

[133] A. Bartha, H.A.H. Billiet, L. De Galan, G. Vigh. J. Chromatogr. 291 (1984) 91.

[134] A. Bartha, G. Vigh, H.A.H. Billiet, L. De Galan. J. Chromatogr. 303 (1984) 29.

[135] A. Bartha, G. Vigh, H. Billiet, L. De Galan. Chromatographia 20 (1985) 587.

[136] A. Bartha, G. Vigh. J. Chromatogr. 395 (1987) 503.

[137] A. Bartha, G. Vigh. J. Chromatogr. 485 (1989) 383.

[138] A. Bartha, G. Vigh. J. Chromatogr. 485 (1989) 403.

[139] A. Bartha, G. Vigh, J. Stahlberg J. Chromatogr. 506 (1990) 85.

[140] T. Welsch, H. Frank, G. Vigh. J. Chromatogr. 506 (1990) 97.

[141] G. Vigh, Z. Varga-Puchony, J. Chromatogr. 196 (1980) 1.

[142] B. Karger, J.R. Gant, A. Hatkopf, P.H. Weiner. J. Chromatogr. 128 (1976) 65.

[143] H. Colin, G. Guiochon, Z. Yun, J.C. Diez Masa, J. Jandera. J. Chromatogr. Sci. 21 (1983) 179.

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